University of Alberta

Single-Strand DNA Ends in Recombination *In Vivo*

by

Haide Razavy ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Molecular Biology and Genetics

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"Far better it is to dare mighty things, to win glorious triumphs, even though checkered by failure, than to rank with those poor spirits who neither enjoy much nor suffer much because they live in the gray twilight that knows neither victory or defeat."

Theodore Roosevelt

This thesis is dedicated to my beloved uncle, Hossein Kafaie Razavy.

ABSTRACT

E. coli possesses more than one pathway of homologous recombination. In wild-type cells, the RecBCD pathway of recombination operates using the RecBCD recombinase. RecBCD recognizes Chi, a recombination hotspot sequence in DNA. Recognition of Chi by RecBCD leads to exchanges at Chi and downstream from Chi in the RecBCD travel path. Several models have been proposed to explain how Chi-stimulated recombination occurs. The split-end model postulates that DNA is unwound at Chi *in vivo* as a result of RecBCD's recognition of Chi. The unwinding is proposed to yield single-stranded DNA with both 5' and 3' ends; both of which are postulated to initiate recombination events by strand invasion of a homologous duplex. In this thesis, evidence that both 5' and 3' ends are formed at Chi *in vivo* will be presented.

E. coli lacking RecBCD recombines DNA poorly, but can become recombination-proficient by acquiring mutations in the *sbcC* and the *sbcB* genes. This alternate pathway of recombination is called the RecF pathway. Here I report that the *sbcB* phenotype is not a null phenotype and provide support for a model in which SbcB protein allows recombination by protecting linear single-strand DNA from degradation.

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1. INTRODUCTION

An organism's ability to reproduce itself relies on the faithful transmission of its genetic material to its offspring. Yet, this information is often rearranged or shuffled before being passed on. This shuffling of DNA, defined as genetic recombination, is necessary for proper segregation of chromosomes in meiosis in eukaryotes; aids in the repair of damaged DNA; assists in the creation of genetic diversity among members of a species; and is a useful tool in applied and basic genetic research. (Leach 1996; Lloyd and Low 1996).

A precise exchange of information between two homologous DNA sequences is defined as homologous genetic recombination. Precise exchanges means that there is with no net gain or loss of participating molecules, due to intermolecular base pairing (Clark 1971; Porter 1988).

Major contributions to the study of homologous genetic recombination have come from work in fungi, bacteria, and bacteriophages (Stahl 1979). *Escherichia coli*, an enteric bacterium, has been used as a model system to study recombination for many decades. Consequently, much is known about the *E. coli* recombination systems (Clark and Sandler 1994; Kowalczykowski *et al.* 1994; Lloyd and Low 1996). Some *in vivo* assays used to study homologous recombination in *E. coli* are the following: conjugational recombination involving Hfr crosses; bacteriophage P1 transductional crosses; and λ lytic crosses in which the λ main recombination proteins are absent (Kowalczykowski *et al.* 1994; Mahajan 1988; Smith and Stahl 1985). In these assays, the *E. coli* recombination machinery acts upon the DNA substrates.

This thesis study focuses on the structure of DNA intermediates in recombination mostly in λ lytic crosses that use the *E. coli* main pathway of recombination, the RecBCD pathway, and an alternative pathway of recombination, the RecF pathway.

Life cycle of bacteriophage λ: (See Fig. 1-1). Bacteriophage λ injects its chromosome of 48.5 kb into an *E. coli* cell, and the two ends of the linear molecule join at *cos* (*co*hesive end site) overhang sequences. After production of the λ replication proteins, using the host protein synthesis machinery, the circular λ chromosome replicates *via* the theta (θ) mode (Furth and Wickner 1983) producing λ monomer circles. At some point, a switch from the theta mode of replication to the sigma (σ), rolling circle mode of replication is made (Furth and Wickner 1983). This switch requires the Gam protein of λ. Gam prevents the *E. coli* double-strand-dependent exonuclease, ExoV (RecBCD), from degrading the linear tail of the rolling circle intermediate (Furth and Wickner 1983; Weinstock 1987). Meanwhile, other λ genes produce proteins needed for the structural parts of λ, the head and the tail (Friedman and Gottesman 1983). Terminase, an important packaging protein, recognizes and binds to the λ packaging origin, *cos*, and also attaches itself to the phage head (Feiss and Becker 1983). Terminase makes a double-strand endonucleolytic cleavage at the bound *cos*, then directs the λ DNA into the capsid, and cuts at the next *cos* site, completing the packaging of the DNA. Only those chromosomes that contain two uncut *cos* sites, one on either side of the molecule, are packaged. Therefore λ monomers cannot be packaged because they contain only one uncut *cos* site. However, dimers and multimers, formed

by rolling circle replication, are eligible for packaging. If Gam is absent, rolling circle replication is prevented by RecBCD nuclease and packageable DNA substrates are few. However, some λ chromosomes can be packaged if monomers recombine with other monomers yielding dimers with two *cos* sites (Fig. 1-1). Either one of the chromosomes in the dimer, but not both of them, can be packaged (Feiss and Becker 1983; Weinstock 1987). The recombination that leads to packaging can occur using the *E. coli* homologous recombination machinery or the λ homologous recombination machinery, the *red* system (Smith 1983; Weinstock 1987). In this thesis study, all the phage used are mutant for *red* and *gam* therefore λ DNA is susceptible to the *E. coli* Rec proteins (Myers and Stahl 1994; Smith and Stahl 1985). Because packaging in λ *red gam* strains requires homologous recombination (Myers and Stahl 1994), this poses a problem when calculating frequencies of λ x λ recombination. A method used earlier to bypass this problem has been used and will be addressed in the appropriate sections (Thaler *et al.* 1989). Measuring the distribution of recombination events along λ DNA can be carried out in λ *red gam* phage (Smith and Stahl 1985). Both types of measurements are presented in this thesis.

Basic steps of homologous genetic recombination: (See Fig. 1-2). Almost all homologous recombination processes can be divided into three main stages: pre-synapsis, synapsis and post-synapsis (Clark and Sandler 1994 and see Kowalczykowski *et al.* 1994; Leach 1996; Lloyd and Low 1996). During the pre-synapsis stage, double-stranded DNA becomes processed, with the help of exonucleases, endonucleases, and/or helicases, into

structures that have protruding single-strand ends. These ends are capable of invading a homologous duplex DNA molecule with the help of proteins that recognize homologous regions and initiate strand exchange. The invasion step is referred to as the synapsis stage. As a result of invasion, cruciform structures known as Holliday junctions can be formed between participating molecules. Holliday junctions are capable of moving to the left or right; this movement is called branch migration. Branch migration requires the help of specific proteins to create heteroduplex DNA, DNA with strands from the two different parental molecules.

Resolution of the recombination intermediate involves the cutting and rejoining of the strands. Cutting those involved in Holliday junction formation gives rise to patch recombinants. Cutting the complementary strands opposite the Holliday junction gives rise to splice recombinants. The steps of branch migration and resolution, following synapsis, is referred to as post-synapsis.

The *E. coli* RecBCD pathway of recombination: We can add details to the generic model discussed above by providing specific information that is known for the wild-type *E. coli* recombination system. Different proteins are involved in each step of the recombination process. Certain DNA sites are important for recombination as well. The DNA sites and key proteins that are involved in the wild-type *E. coli* recombination pathway (RecBCD pathway) include the following: RecA, RecBCD, Chi (χ), and double-strand breaks as well as proteins for Holliday junction processing, RuvABC, or RecG.

RecA: RecA is vital for recombination (see Clark and Low 1988; Kowalczykowski 1991; Kowalczykowski *et al.* 1994; Radding 1988). *recA* defective cells recombine 10^5-fold less efficiently than wild-type cells. *In vitro*, RecA coats single-stranded DNA, and helps it to invade a duplex recipient (reviewed by Roca and Cox, 1997). RecA helps the invading strand find a homologous region within the recipient duplex, and once this homology is found, RecA catalyzes strand exchange between the invading and recipient molecules.

RecBCD: RecBCD is a heteromultimeric enzyme with subunits encoded by the *recB*, *recC*, and *recD* genes (Kowalczykowski *et al.* 1994; Taylor 1988). RecBCD is a powerful double-strand exonuclease (ExoV) which degrades linear DNA to small oligonucleotides. RecBCD also possesses a helicase activity *in vitro*. *recBC* mutants recombine 10-1000 fold less efficiently than wild-type cells which indicates that RecBCD is also a recombination protein. *recD* mutants, like *recBC* mutants, are nuclease-deficient. However, unlike *recBC* cells, *recD* mutants are hyper-recombinagenic. It is proposed that the RecD subunit represses the recombination-promoting activity of RecBCD (Thaler *et al.* 1989). Data accumulated over the past nine years suggest that the removal of the RecD subunit is regulatory in that it promotes the helicase (and hence the recombinagenic activity) of the RecBCD enzyme over the nuclease activity. RecBCD also possesses ATP-dependent single-strand exonuclease and ATP-stimulated single-strand endonuclease activities *in vitro*.

Chi: Chi (*c*rossover *h*otspot *i*nstigator) is a DNA sequence (5'GCTGGTGG3') that elevates recombination five to ten-fold above the level seen in Chi-free DNA (for reviews see Kowalczykowski *et al.* 1994; Rosenberg and Hastings 1991; Smith and Stahl 1985). Chi stimulates recombination at, and to the left of itself on the standard λ map, extending as far as 10 to 20 kilobasepairs away. Chi was first noticed as a mutation that increased the plaque size of λ mutants that were defective in their *red* recombination system and in *gam* (Henderson and Weil 1975; Lam *et al.* 1974). *gam* mutants cannot replicate *via* rolling circle (see above), and because multimeric DNA is required for packaging, the only other route for a phage burst is through recombination using the host RecBCD system. Recombination using RecBCD produces small phage bursts (evidenced by the small plaque sizes) unless λ carries a Chi mutation which stimulates recombination.

RecBCD physically contacts Chi and this action leads to recombination. Because Chi is present in the *E. coli* chromosome about once every 5 kb, it is probably responsible for most of the RecBCD-mediated recombination events on the chromosome.

Stimulation by Chi is orientation-dependent. In λ, RecBCD loads onto the double-strand end that is created when terminase cleaves at *cos*, the λ packaging origin. Because terminase remains bound to the left end of the λ DNA, RecBCD can load only from the right side. If Chi is oriented the proper way, the GG3' side to the right end, RecBCD can recognize Chi and recombination can take place. If Chi is inverted, it does not promote recombination because RecBCD is incapable of recognizing it. However, if RecBCD travels from the left end of the λ chromosome (inverted *cos*), the

inverted Chi is recognized and recombination takes place. These observations indicate that recombination promoted by Chi is dependent on another asymmetric DNA sequence, *cos*.

Double-strand ends: Double-strand breaks occur at *cos* and are important for recombination *via* the RecBCD pathway because RecBCD can load only onto a duplex DNA end *in vitro* and *in vivo* (Stahl *et al.* 1983). Double-strand ends are needed for RecBCD-mediated recombination in other assays. For example, in conjugational recombination, the replicated donor DNA is linear and accordingly becomes a substrate for RecBCD action. In transductional recombination, the donor DNA is also linear and double-stranded. Double-strand breaks in the DNA may occur during replication of the *E. coli* chromosome (Michel *et al.* 1997). RecBCD may load onto replication-induced double-strand breaks for recombinational repair (Kuzminov, 1995). Therefore much of the recombination may occur *via* double-strand ends, the loading site for the RecBCD recombination enzyme.

Other proteins such as branch migration proteins, Holliday junction resolvases, and single-strand binding proteins are needed for the RecBCD pathway of recombination to function (Clark and Sandler 1994; Kowalczykowski *et al.* 1994). However these proteins are not the focus of this thesis and will not be dealt with in detail here.

How do all the players of the RecBCD pathway described above interact with each other and/or the DNA to effect recombination? Several models have been proposed but two will be discussed in detail here (Rosenberg

and Hastings 1991; Smith *et al.* 1981). The two models differ in the following respects: (1) the mode of travel of the RecBCD enzyme from the loading site to Chi, (2) the enzymatic activity of the RecBCD recombinase that promotes recombination, and (3) the structure of the Chi-stimulated products recovered (Rosenberg and Hastings 1991). The first (earlier) model is the following:

Nick-initiation model for Chi: (See Rosenberg and Hastings 1991; Smith *et al.* 1984; Smith *et al.* 1981; see Fig. 1-3). The nick-initiation model for Chi activity, proposed by Smith and colleagues, was a popular model that explained many of the observations obtained at that time. The model made specific predictions for the structure of heteroduplex products, patches, that are formed as a result of Chi-stimulated recombination. The nick-initiation model proposes that RecBCD enters DNA at a double-strand break and travels as a helicase along the chromosome, from right to left, unwinding strands ahead of it and rewinding the strands behind it. [The helicase activity of RecBCD can be obtained *in vitro* with the addition of Ca^{2+}. Under these conditions, the degradative nuclease activity of RecBCD is inhibited]. Occasionally, RecBCD nicks at the strand that ends 3' at the end of the molecule at which RecBCD loaded. When RecBCD encounters Chi from the GG3' side, it pauses and nicks a few nucleotides upstream of Chi. As the RecBCD enzyme continues unwinding the DNA past Chi, a single-stranded 3' end is formed. The 3' end is coated with RecA and strand invasion ensues to produce a Holliday junction. The recombination intermediate is resolved either into patches or splices.

The nick-initiation model predicts that only 3' end invasions occur. Consequently, the patch recombinants of such an event should have the recombinant information exclusively in the strand ending 3' where RecBCD loaded, the *l* strand of λ (Rosenberg 1987).

Hagemann and Rosenberg (1991) tested this prediction and found that their results did not fit with the nick-initiation model. Some of the patches they studied contained recombinant information in the 3'(*l*) strand, but twice as many contained recombinant information in the 5'(*r*) strand. Because both *l*-strand and *r*-strand patches were observed as products of recombination (Hagemann and Rosenberg 1991), both 3' and 5' ends were proposed to be capable of invading a DNA molecule (Rosenberg and Hastings 1991). Invasion might not be exclusive to the 3' ends.

Split-end model for Chi-stimulated recombination: (See Figure 1-4). Rosenberg and Hastings (1991) proposed a model, incorporating the strand patch results as well as a series of more recent observations, to explain how Chi-stimulated recombination works. The split-end model proposes that RecBCD enzyme loads onto DNA at a double-strand end as ExoV. It then travels as a degradative double-strand exonuclease, unwinding and nicking DNA strands as it goes. Upon encountering Chi from the GG3' side, the RecBCD nuclease loses its RecD subunit and continues unwinding as a helicase, but with no nuclease activity. The helicase function of RecBC(D^-) is proposed to be the recombinagenic activity that allows it to unwind DNA ends at Chi, as it continues to travel leftward, to produce single-stranded 3' and 5' ends. Both strands are proposed to be

invasive after being coated with RecA protein, and to give rise to both 3'-strand patches and 5'-strand patches.

The split-end model has been supported by recent work. Dixon *et al.* (1993) reported that *in vitro* DNA is degraded from the RecBCD loading site until Chi, and that once RecBCD contacts Chi, it begins to unwind the DNA. Taylor and Smith (1995) have found conditions in which RecBCD forms the split-end intermediate *in vitro*. *In vivo* experiments also support the degradation step (Stahl *et al.*, 1990; Dabert *et al.* 1992).

The hypothesis that the loss of the RecD subunit switches the nuclease mode of RecBCD into a helicase mode is supported by the following observations: *recD* null mutants lack Exonuclease V activity, but are hyper-recombinagenic (Biek and Cohen, 1986; Amundsen *et al.* 1986). Recombination in *recD* cells depends on functional RecB and RecC proteins (Amundsen *et al.* 1986; Thaler *et al.* 1989). This means that the RecBC enzyme is a better recombinase than a nuclease in the absence of RecD. Also, exchanges in *recD* cells are clustered at double-strand breaks, as if the RecBC enzyme is already in its recombinase mode before even reaching Chi (Thaler *et al.* 1989). Finally, the distribution of exchanges seen in *recD* cells are superimposable on the distribution of exchanges in wild-type cells when Chi is located close to RecBCD's loading site (Thaler *et al.* 1989).

The split-end model proposes that the helicase activity of RecBC(D^-), produced by interaction with the Chi sequence, produces both 5' and 3' single-strand ends. The possibility of both 5' and 3' single-strand invasion was established by Dutreix *et al.* 1991, who showed that both 5' and 3' ends are capable of undergoing RecA-mediated strand exchange with a

homologous DNA molecule. Rinken *et al.* (1992) demonstrated that RecBC(D^-) is capable of unwinding *in vivo* to create single-stranded 5' and 3' ends.

In one portion of this thesis study, *in vivo* experiments were performed to test whether both 5' and 3' single-strand ends exist at Chi (as predicted by the Split-End model). Chapter 2 of this thesis presents evidence supporting this idea. In Chapters 3, 4, and 5, extensions of the work presented in Chapter 2 are carried out.

The *E. coli* RecF pathway of recombination: The RecF pathway is used by *E. coli* when the cell lacks RecBCD enzyme and gains mutations in the *sbcC* gene (Lloyd and Buckman 1985) and a particular mutation in the exonuclease I gene, *sbcB15* (Kushner *et al.* 1971). Wild-type Exo I is a 3' single-strand-dependent exonuclease, and wild-type *sbcC* encodes an ATP-dependent double-strand-dependent exonuclease (Connelly and Leach 1997; Kushner *et al.* 1971) In *recBC* cells, recombination drops 10^2-10^3 fold, and sensitivity to DNA-damaging agents, UV radiation and mitomycin C, is observed (Emmerson 1968; Willets and Mount 1969). *sbcB15* and *sbcC* mutations restore recombination and repair proficiency to a *recBC* strain (Kushner *et al.* 1971; Lloyd and Buckman 1985). Other alleles defective for ExoI, called *xonA*, suppress repair deficiency but not recombination deficiency (Kushner *et al.* 1972). Although attempts have been made to sort out the difference between the two classes of ExoI-deficiency mutations, *sbcB15* and *xonA*, the reason why only *sbcB15* mutations restore recombination was not known. My construction of a null

mutation in the Exo I-encoding gene permitted us to explore this question. Studying the nature of *sbcB15* suppression can also provide clues to the structure of recombinational intermediates in the RecF pathway. The genetic characterization of the *sbcB15* mutation in both the RecBCD and RecF pathways is presented in Chapter 6.

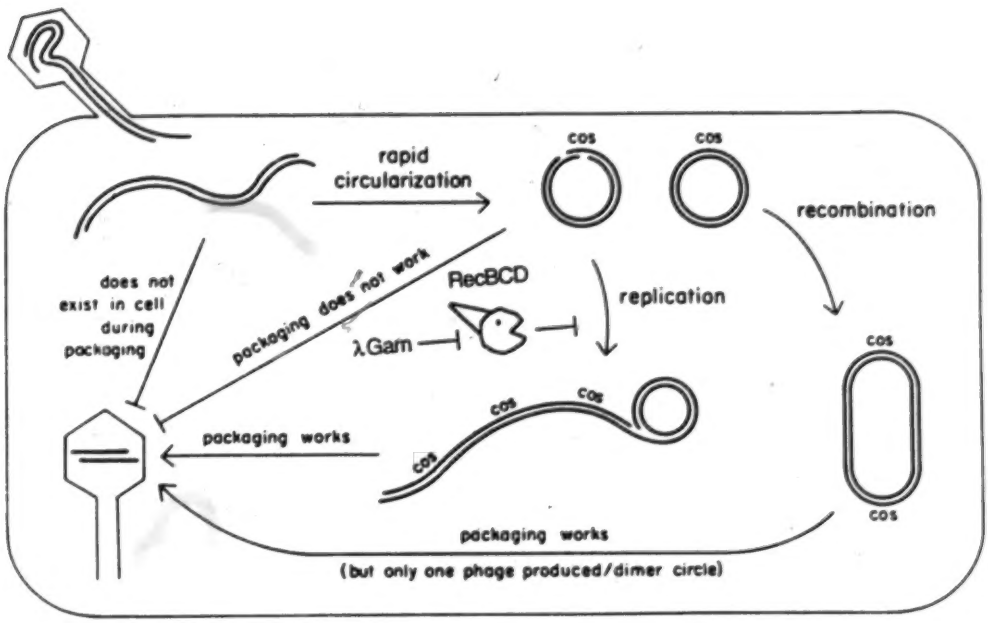

FIGURE 1-1.—Life-cycle of bacteriophage λ. This figure is modified from Rosenberg *et al.* 1985)

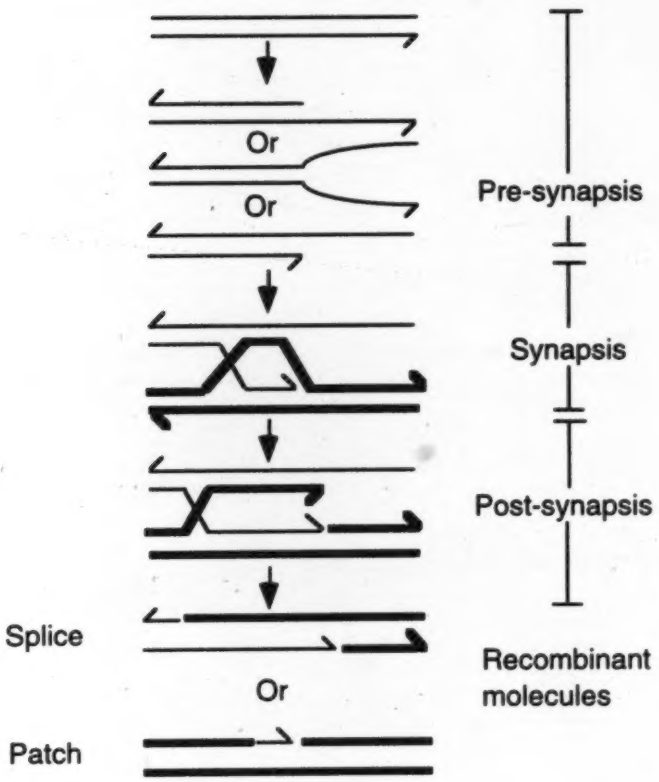

FIGURE 1-2.—Basic steps of homologous recombination (discussed in text).

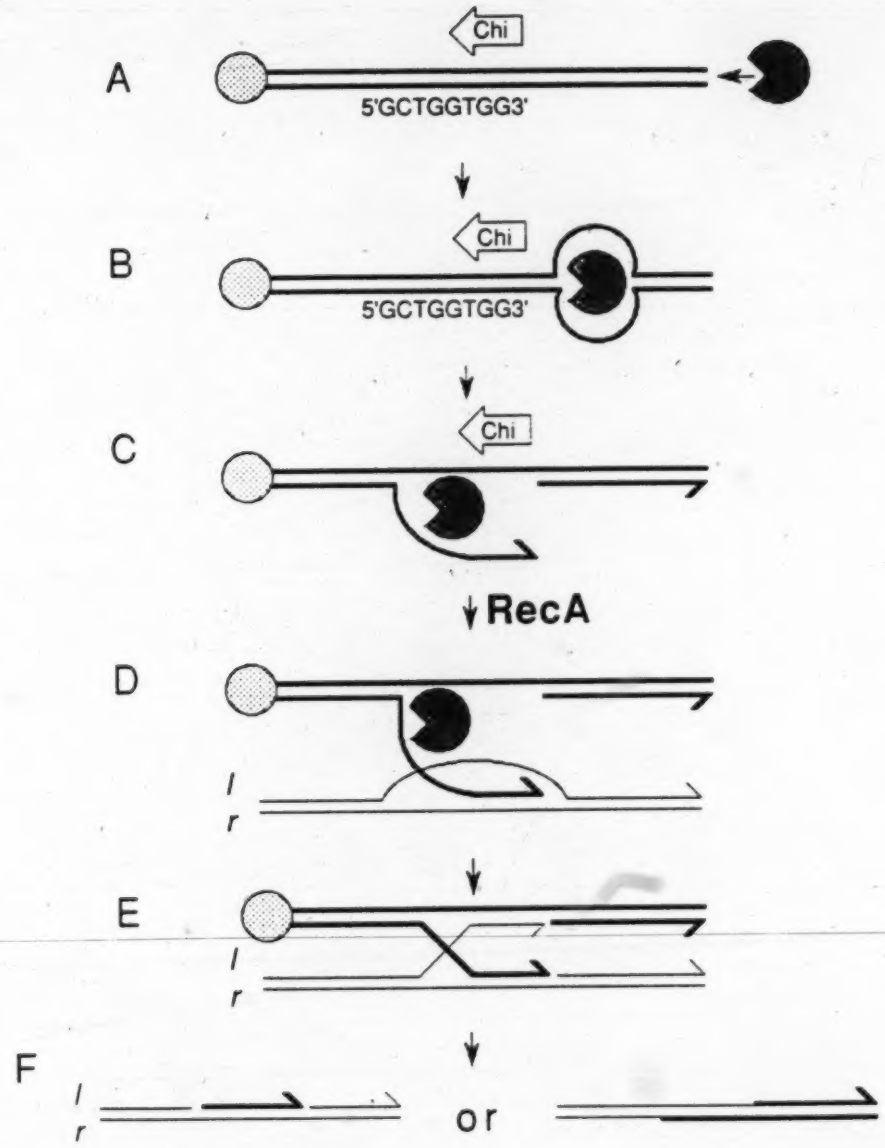

FIGURE 1-3.—Nick-initiation model for Chi (discussed in text). Figure taken from Rosenberg and Hastings (1991).

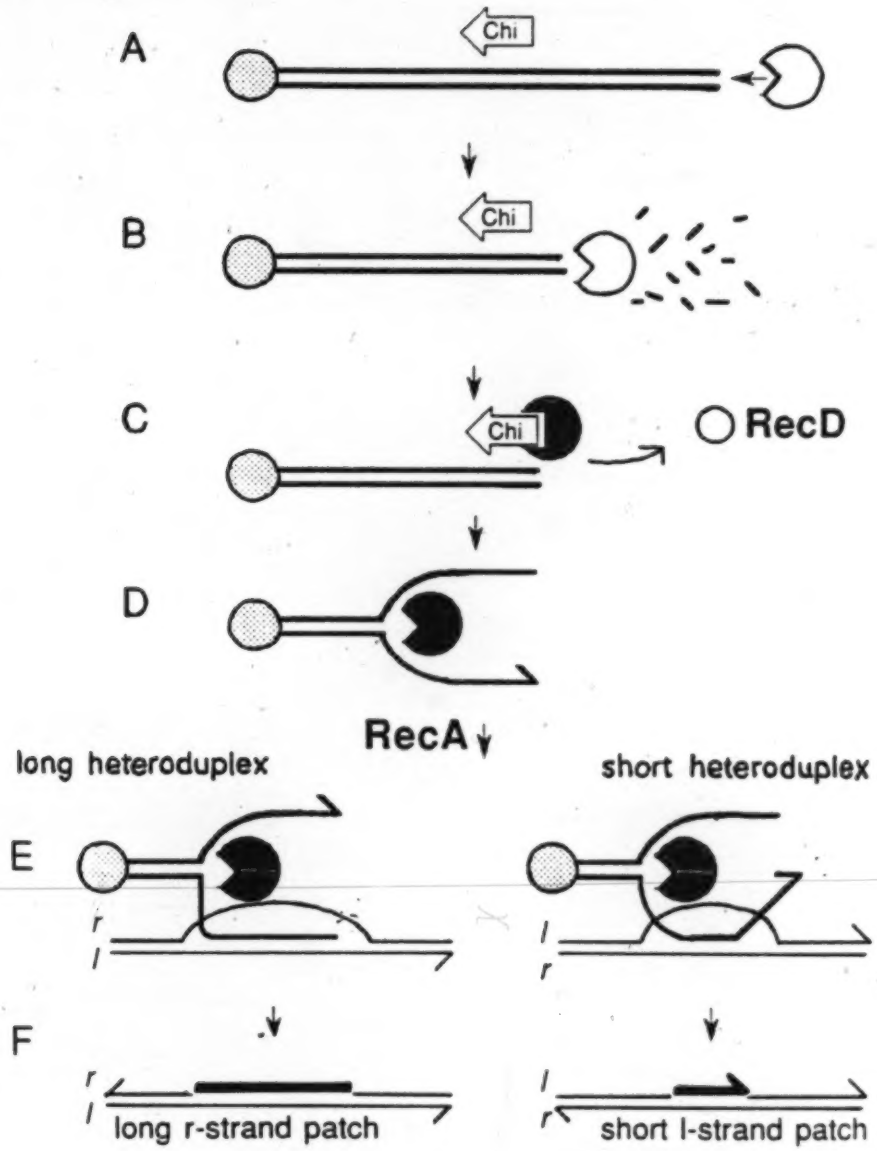

FIGURE 1-4.—Split-end model for recombination at Chi (discussed in text). Figure taken from Rosenberg and Hastings (1991).

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2. EVIDENCE FOR BOTH 3' AND 5' SINGLE-STRAND DNA ENDS IN INTERMEDIATES IN CHI-STIMULATED RECOMBINATION *IN VIVO*.*

Chi sites enhance the productivity of RecBCD-mediated homologous recombination (see Kowalczykowski *et al.* 1994; Myers and Stahl 1994). Chi is a small sequence, 5'GCTGGTGG, that interacts directly with the RecBCD recombination enzyme of *Escherichia coli* to promote recombination locally. RecA protein is required in the reaction, as are the *E. coli* Holliday junction resolution systems (Lloyd 1991; West 1994). Also, a DNA double-strand break (DSB) must occur in one of the recombining molecules for RecBCD enzyme to load onto the DNA and then interact with Chi (Taylor, 1988). The DSB can be many kilobasepairs distant from Chi, but RecBCD travels from it to Chi and recombines DNA preferentially near Chi.

Several different molecular mechanisms by which the components listed above could interact to produce recombinant product DNAs *in vivo* have been considered (*e.g.*, Smith *et al.* 1981; Rosenberg 1987, 1988; Stahl *et al.* 1990; Dixon and Kowalczykowski 1991,1993; Hagemann and Rosenberg 1991; Rosenberg and Hastings 1991). Many of them have in common the use of single-strand DNA ends as intermediates in recombination. An example of such a model is shown in Figure 2-1. In it, single-strand DNA ends of both 3' and 5' polarities are formed at Chi, and both of these generate recombination products. This model makes use of RecA protein's ability to catalyze strand exchange with 3'- and 5'-ended single strands *in vitro* (Dutreix *et al.* 1991). It has the unusual feature that ends of different polarities can substitute for each

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other in recombination with either a 3' or a 5' single-strand end being sufficient.

Although single-stranded DNA ends are believed widely to occur in intermediates in recombination, such intermediates have not yet been documented to form *in vivo* during recombination at Chi. In this paper, we offer evidence for the existence of single-strand DNA ends in intermediates during some stage of recombination *in vivo*. We find that recombination is affected by nucleases that are specific for single-strand DNA ends and that can digest ends of both polarities. This implies that both 3' and 5' single-strand ends occur in intermediates in Chi-stimulated recombination.

MATERIALS AND METHODS

A null mutation in the gene encoding Exo I was constructed by *Mlu*I-*Xma*I digestion of the plasmid pDPK20 (Philips and Kushner 1987) to remove a 630-bp fragment of the gene, which was replaced with the chloramphenicol acetyl transferase (CAT) gene cassette in a 908-bp *Bsp1286*I-*Xma*I fragment of plasmid pCAT19 (Fuqua 1992). The *Mlu*I and *Bsp1286*I sites are linked by the sequence CGCGTCTCGAGGGGCA which was added as a double-strand oligonucleotide with the first four and last four bases shown overhanging as single-strand ends. This new deletion-substitution allele is named Δ*xonA300::Cat*. The plasmid bearing this allele, pHR1, was digested with *Cla*I and *Hpa*I to release a 3.8-kb fragment containing the disrupted *xonA* gene. This fragment was used for targeted replacement of the wild-type *xonA* gene in a *recD* strain, as described by Russell *et al.* (1989), with selection for chloramphenicol resistance. The chloramphenicol-resistance cassette's proper

insertion in *xonA* was verified by P1 transductions of the allele that demonstrated linkage with the nearby *his4* gene, and the *xonA* gene itself. Other single-strand exonuclease defective mutations used are *recJ284*::Tn*10* (Lovett and Clark 1985), *sbcB15* (Kushner *et al.* 1971), and Δ*(xseA-gua) zff3139*::Tn*10Kan* (Vales *et al.* 1979). All bacterial strains used as cross hosts in this work are derivatives of AB1157 (B. Bachmann) and all are Su^-. JC11450, a Su^- derivative of AB1157, (A. J. Clark, Berkeley) is Exo^+ (lab collection number SMR122). JC11451(A. J. Clark, Berkeley) is *sbcB15* (SMR123). Derivatives of JC11450 are *recJ284*::Tn*10* (SMR124), Δ*xonA300*::*Cat* (SMR839), and Δ*xonA300*::*Cat recJ284*::Tn*10* (SMR1403). Spontaneous Su^- derivatives of the AB1157 (SuII) strains obtained from S. Lovett, Brandeis University, were constructed by S. K. Szigety: Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR784; parent STL1605), *recJ284*::Tn*10* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR 774; parent STL1522), and *sbcB15 recJ284*::Tn*10* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR775; parent STL1607). Δ*xonA300*::*Cat* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR1262) and Δ*xonA300*::*Cat recJ284*::Tn*10* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR1404) were constructed from SMR784.

All bacterial strains were constructed by standard P1 transduction methods. Phage λ strains are from our laboratory collection. Phage crosses and cesium formate density gradient centrifugation were performed as described previously (Rosenberg 1987; Thaler *et al.* 1989)

RESULTS AND DISCUSSION

We wished to examine the idea that single-strand DNA ends of both polarities occur in intermediates in recombination at Chi sites (*e. g.* ,Figure 2-

1). *E. coli* possesses single-strand-specific exonucleases of both polarities: Exo I is 3'-specific, RecJ is 5'-specific, and Exo VII digests single-strand ends of both polarities (see Linn *et al.* 1993). If 5' and 3' single-strand ends exist in recombination intermediates at Chi, then these exonucleases might affect recombination there.

Single-strand-dependent exonucleases of 5' and 3' polarity facilitate Chi action opposite heterologous DNA: The first test of this idea involves the ability of Chi sites to stimulate recombination efficiently when opposite a large heterologous DNA sequence (Stahl and Stahl 1975). Such recombination occurs downstream of the heterology, distant from the DSB site at which RecBCD loads. If single-strand DNA ends form at Chi (*e. g.* ,Figure 2-1 D) and those ends are necessary for recombination, then perhaps those ends must be degraded past the heterology for efficient recombination downstream in the homologous region.

Phage λ crosses diagrammed in Figure 2-2 assess the ability of Chi to act opposite a heterology. The ratio of recombinants in the downstream interval that Chi stimulates is compared with a control interval unaffected by Chi, in crosses with Chi opposite heterologous or homologous DNA. The ratio of recombinants in each interval or "hotspot value" (Table 2-1) provides a quantitative measure of Chi activity.

The Chi hotspot crosses diagrammed in Figure 2-2 were performed in cells proficient or deficient for all three single-strand exonucleases listed. The data in Table 2-1 show that when the three exonucleases are absent, Chi works only about half as well when it is opposite a heterology. The nucleases do not affect the Chi hotspot value when Chi is opposite homologous DNA.

This implies that the single-strand ends that are being affected by these nucleases occur at the Chi site. That is, for the exonucleases to "sense" the difference between homology- and heterology-containing DNA at Chi, presumably they must be acting on the DNA in that region.

Interestingly, single-strand exonuclease single mutants and double mutants show little effect on the Chi activity opposite a heterology (Table 2-1). It is as if these nucleases can substitute for each other, such that all three must be removed to see the effect. This suggests that the substrates of these nucleases, the 3' and 5' single-strand ends, can substitute for each other as intermediates in recombination, such that both polarities of exonuclease must be absent to inhibit recombination opposite a heterology. An alternative interpretation is considered below (Further Discussion).

One way to understand these results is in the context of the model in Figure 2-1 in which single-strand DNA ends form at Chi. It could be that for the most efficient propagation of Chi-stimulated recombination downstream of the heterology, these ends must be degraded by the single-strand-dependent exonucleases until single-strand ends homologous with the partner molecule are exposed. This could occur before or after RecA-mediated homologous pairing (discussed below). In any model, the importance of these results is in the indication that particular DNA substrates: single-strand DNA ends with 5' and 3' polarity, exist at Chi *in vivo* and are involved with recombination.

The *sbcB* gene product behaves differently from a simple absence of Exo I: The exonuclease mutations we used are null mutations. We constructed the null allele of *xonA*, the gene encoding Exo I (Materials And Methods), because other alleles of this gene available in *E. coli* are probably not null

alleles (*e. g.* ,Philips *et al.* 1988; Benson and Roth 1994). *sbcB15*, a putative partial-function allele (*e. g.* ,Philips *et al.* 1988; Benson and Roth 1994), has been used widely. We find that it does not behave as the deletion allele does (Table 2-2). *sbcB15* depresses Chi activity even when the other single-strand-dependent exonucleases are present, as if the SbcB15 protein blocks the action of those nucleases on DNA (also H. Razavy, R. Sidhu and S. M. Rosenberg unpublished results). This makes *sbcB15* unsuitable for tests of the polarity of the DNA ends involved in recombination *in vivo*.

Myers *et al.* (1995) used *sbcB15* and *recJ* mutations in crosses similar to ours and found evidence for 3' but not 5' single-strand ends participating in Chi-stimulated crossovers *in vivo*, in apparent disagreement with our results (Table 2-1). The results presented here imply that these authors failed to detect an effect of RecJ nuclease, and thus 5' single-strand ends, because of the masking effect of the SbcB15 protein (Table 2-2). This is not observed when a null mutation is used instead and when all three single-strand exonucleases are eliminated (Table 2-1).

Both 5' and 3' single-strand-dependent exonucleases affect the frequency of recombination: The hotspot crosses (above) measure the distribution of recombination events, *i. e.* ,where recombination occurs, but do not address how much recombination occurs. We also measured frequencies of Chi-stimulated recombination in the absence of single-strand exonucleases. A problem in such measurements is that every λ molecule must dimerize *via* recombination in order to become packageable, because monomeric λ DNAs cannot be packaged (see Myers and Stahl 1994). Therefore every progeny particle contains a recombined λ chromosome. As done previously (Thaler *et al.* 1989), we have measured frequencies of recombination by providing an

alternative route to packageability. The phage's site-specific recombination system, Int, can recombine two λ molecules at the *att* site only (Figure 2-3). We isolated such site-specific recombinants and then assayed the frequency of a second, gratuitous homologous recombination event as diagrammed in Figure 2-3. The site-specific recombinant carries neither of two net deletions present in the parental molecules (labeled Δ and *bio1* in Figure 2-3). This allows it to be isolated in cesium formate equilibrium density gradients of the cross progenies as the densest peak of the gradients (Thaler *et al.* 1989). From that peak the percentage of phage that experienced homologous recombination events was determined (Figure 2-3).

The data in Figure 2-3 show that removal of single-strand exonucleases of both polarities decreases the amount of homologous recombination by about four-fold. The exonuclease mutations cause no such pattern, positive or negative, on the yield of Int-promoted recombinants in these crosses (data not shown). Each bar in the graph represents data from a separate cross and density gradient, with 16 density gradients represented in Figure 2-3. When removed singly, each single-strand exonuclease affects the frequency of recombination only slightly, but removal of both 5' and 3' single-strand exonucleases depresses recombination significantly. The RecJ (5') plus Exo I (3') nucleases cause the largest effect with Exo VII contributing only slightly more. This supports the conclusion (above) that either 5' or 3' single-strand ends can be used as recombination intermediates, resulting in the observation that the exonucleases of opposite polarities can substitute for each other in recombination.

These results also imply that some degradation of 5' and 3' single-strand ends facilitates recombination. L. Miesel and J.R. Roth (1996) observe a

similar effect with removal of RecJ and Exo I in Salmonella transductional crosses. Their depression of recombination increases to 30-fold when the length of recombining DNAs is made very small. They, and we, suggest the following interpretation of these results. It could be that the split-end intermediate (shown in Figure 2-1 D) may sometimes reanneal. This back reaction to the intermediate shown in Figure 2-1 C, would eliminate single-stranded DNA ends (Rosenberg and Hastings 1991). Digestion of one or the other single-strand DNA end illustrated in Figure 2-1 D would make the lengths of the two strands unequal (Figure 2-1 E) so that one of the two strands would remain single-stranded, and able to recombine, even if reannealing occurred. A small effect of a *recJ* mutation on conjugational recombination in *recD* cells has been reported previously (Lovett *et al.* 1988). Their finding implies the existence of 5' single-strand DNA ends in the context of RecBC-mediated conjugational recombination in the absence of RecD.

The experiments in Figure 2-3 measure recombination in the phage λ left arm stimulated by a Chi, *Chi-bio*. Similar results are seen when recombination in λ right arm is measured, and when a different Chi, *ChiC* is used, and when no Chi is used (Figure 2-4). Dark bars represent *ChiC*-stimulated recombination, and light bars show recombination in the absence of Chi. The increased height of the dark bars (Chi^+) relative to the light bars (Chi°) reflects the extent to which Chi stimulates recombination. *Chi-bio* gives a slightly smaller overall percent of recombination (~12-13%, "Exo^+" bars in Figure 2-3) than *ChiC* (~16-17%, "Exo^+" dark bar in Figure 2-4), probably because of the somewhat different length of the recombining region in the two crosses. Both Chi^+ and Chi° recombination levels decrease in

single-strand nuclease defective strains. As in Figure 2-3, the largest effect comes from removal of RecJ (5') and Exo I (3'), with Exo VII (5' and 3') contributing little.

FURTHER DISCUSSION

The experiments described here are directed at asking whether specific structures of DNA intermediates occur in recombination *in vivo*. The approach used is to test whether the ability to recombine DNA is affected by the presence of enzymes that are specific for those DNA substrates, at least *in vitro*. To cope with the possibility that any of the single-strand specific exonucleases used could have a different substrate specificity *in vivo*, we have used three different enzymes all of which are described as single-strand dependent exonucleases, biochemically, and we find that they behave similarly to each other *in vivo*. This congruence implies that the enzymes are single-strand specific exonucleases *in vivo* as well, and indicates that single-strand DNA ends, the substrates of these enzymes, occur *in vivo* in DNA molecules on a pathway leading to recombinant products.

The depressions of recombination are partial, *i.e.*, not a complete recombination-deficient phenotype, however the fact that fully three-fourths of recombination is eliminated in the triple exonuclease mutant (Figures 2-3 and 2-4) indicates that a majority of the intermediates present are susceptible to these nucleases. Moreover, it is probable that the recombination activity that persists in the triple mutant could be facilitated by some of *E. coli*'s multiple other nucleases (see Linn *et al.* 1993), which we have not removed by mutation.

Are the effects of these exonucleases in recombination direct, *i.e.*, manifested at the level of the DNA substrate that is recombining? The finding that the nucleases tested affect Chi hotspot values when Chi is opposite a heterology, but not opposite homology (Table 2-1), indicates a direct effect that occurs at the DNA at Chi. It is difficult to imagine how the exonucleases could distinguish the presence or absence of heterologous DNA unless they were acting on the DNA in that region.

The single-strand exonucleases of opposite polarities were found to substitute for each other in recombination in three different experimental protocols. This result implies that the substrates of these enzymes, 3' and 5' single-strand DNA ends, have the ability to substitute for each other in recombination. This finding will be compared with various models for Chi-promoted recombination below.

The single-strand ends that are implicated could occur presynaptically (*e. g.* ,see Figure 2-1, D and E) or post-synaptically (*e. g.*, see Figure 2-1 F) or both. Because the exonucleases used are single-strand-dependent, only single-stranded extrusions from a synapsed structure should be susceptible. For most of the discussion below, we will assume that the ends occur in the invading molecule (thin lines Figure 2- 1F), which is known to be linear, not in the invaded molecule (thick lines Figure 2-1 F), which is thought to be circular, without any DNA ends (see Myers and Stahl 1994). However, the possibility of ends in the invaded molecule will also be considered at the end. The possibility of presynaptic single-strand intermediates will be addressed first.

A key distinguishing feature of models for the molecular mechanism of recombination promoted by Chi sites is whether strand invasion is performed

by single-strand ends of one polarity, 3', (Smith *et al.* 1981; Dixon and Kowalczykowski 1991, 1993) of both polarities (Rosenberg and Hastings 1991), or without the use of single-strand DNA ends at all (Stahl *et al.* 1990). According to the split-end hypothesis (Rosenberg and Hastings 1991, Figure 2-1) free 3' and 5' single-strand DNA ends are formed at Chi and are used as strand-invasion intermediates in recombination. This idea is supported by our finding here that three different enzymes with single-strand dependent exonuclease activity of 3' polarity, of 5' polarity and of both polarities affect RecBCD-mediated Chi-stimulated recombination if these ends occur in the invading molecule. This implies that 3' and 5' single-strand ends are interchangeable as intermediates, a prediction not as readily understood in the context of some other models (Smith *et al.* 1981; Dixon and Kowalczykowski 1991, 1993).

What if a postsynaptic single-strand end (from the invading molecule) is the target of the exonucleases that affect recombination in our assays? In two models that use 3' end invasion exclusively (Smith *et al.* 1981; Dixon and Kowalczykowski 1991, 1993) digestion of the 3' overhanging tail from a strand exchange intermediate could be required to complete recombination, especially when Chi acts opposite a heterology, and the end might require post-synaptic digestion up to the region of homology. However, it is difficult to imagine how a 5'-specific exonuclease could substitute for this requirement. A third model (Stahl *et al.* 1990) specifies intermediates without single-strand character which are not expected to be susceptible to these nucleases before or after synapsis.

What if the exonucleolytic digestion occurs on the invaded, not the invading molecule? The above predictions hold true for digestion of DNA

ends on the invading molecule, an idea that we favor because of the documented presence of ends on the invading molecule and the lack of any evidence for their existence on the invaded molecule (reviewed by Taylor 1988; Rosenberg and Hastings 1991; Kowalczykowski *et al.* 1994; Myers and Stahl 1994). However, if a strand break were to occur in the displaced strand of a D-loop in the invaded molecule (Figure 2-5 B), then expectations for the roles of single-strand nucleases are different. There is no known endonucleolytic function that nicks D-loops in *E. coli* recombination. However, if one were to postulate the existence of such an activity, then single-strand exonucleases could function in removing the displaced strand of the D-loop to facilitate RecA promoted strand exchange. Digestion of the displaced strand facilitates RecA-mediated strand exchange *in vitro* (Konforti and Davis 1992; Bedale *et al.* 1993; Corrette-Bennett and Lovett 1995). Interestingly, such digestion of the displaced strand could use an exonuclease of the same polarity as the DNA end that invaded (Figure 2-5 B). For example, a 3' end invasion intermediate is promoted by 3' digestion of the displaced strand of the invaded molecule (Figure 2-5B), as well as by 5' digestion of the invading molecule (Figure 2-5A).

Does the above consideration mean that one cannot distinguish models using 3' end invasion exclusively from those using both 3' and 5' ends? We think not. Note that models that utilize 3'-end invasion exclusively make the prediction that recombinant products called "patches" (single strand inserts into an otherwise unrecombined molecule, illustrated in Figure 2-5, A and B, bottom) should be specific to the 3' ending strand that invaded, the strand ending 3' at the right end illustrated in Figure 2-5, A and B, and also the phage lambda right end on the conventional map. Hagemann and Rosenberg

(1991) found that patches occur in both strands. This deviates from the expectation of models using 3' end invasion exclusively, and is the impetus for the proposal that both 5' and 3' DNA ends can invade (Rosenberg and Hastings 1991). Regarding whether invading or invaded strand are affected by the single-strand exonucleases, it should be informative to assess whether nucleases of each polarity specifically affect patches of each polarity.

The split-end model (Rosenberg and Hastings 1991, Figure 2-1) anticipates the involvement of single-strand DNA ends of both polarities which can substitute for each other in recombination. This model has received support from other sources. The model postulated that RecBCD is converted from a double-strand exonuclease (Exo V) to the single-strand-producing helicase at Chi by Chi-mediated inactivation of the RecD subunit. Rinken *et al.* (1992) demonstrated that RecD-deficient RecBC enzyme is a helicase *in vivo*. The transformation of RecBCD enzyme into a *recD* phenocopy by Chi first proposed by Thaler *et al.* (1989) has been substantiated (Dabert *et al.* 1992; Kuzminov *et al.* 1994; Zaman and Boles 1994). DNAs with the structures predicted in some of the steps of the model have been isolated from cells (Kuzminov *et al.* 1994). Our results imply that such molecules are *bona fide*, product-generating recombination intermediates and not an unproductive substrate. Recently, Mg^{2+} concentrations have been found under which purified RecBCD generates the split-end intermediate (Figure 2- 1D) at Chi *in vitro* (Taylor and Smith 1995). Further work will be necessary to determine whether 3' and 5' ends give rise to 3' and 5' single-strand heteroduplex patches (Hagemann and Rosenberg 1991), respectively, as was originally suggested (Rosenberg and Hastings 1991).

Because of the many biochemical activities reported for RecA protein and RecBCD enzyme (Taylor 1988; Kowalczykowski *et al.* 1994), the approach used here, to examine the DNA intermediates in whole recombination reactions *in vivo*, is an important complementary strategy. This approach may help elucidate what these enzymes do to DNA during recombination in the cell.

TABLE 2-1—Crosses of the phage described in Figure 2-2 were performed in each of the genotypes of cell indicated (Materials and Methods) the number of times indicated(n). For each set of crosses performed in parallel, the hotspot value was calculated as the ratio of the number of recombinants in the Chi-stimulated interval /number of recombinants in the control interval in a cross with Chi present (Chi^+). This fraction is divided by a similar ratio taken from a cross done in parallel with Chi absent (Chi°) to give the hotspot value (Stahl *et al.* 1983; see Figure 2-2). Hotspot values (presented as means ±SE) of unity would indicate no Chi activity. The extent to which Chi works is seen in the magnitude of the hotspot value greater than one (Stahl *et al.* 1983). Thus each hotspot value represents a set of two phage crosses in a single culture of bacteria, with hundreds to thousands of recombinant plaques scored for each. To compare as many strains as we did with as many different crosses, data from the different sets of crosses done on different days were normalized as follows. The mean hotspot value for Exo^+ was determined. All of the different Exo^+ values were set equal to this mean and the values in the mutant strains were normalized to the Exo^+ value from the cross done in parallel with it. This gives an accurate picture of the relative differences between the different genotypes by removing the variability that would result from averaging absolute values that vary somewhat from day to day, more than the relative differences between the different cell genotypes do.

TABLE 2-1

Ability of Chi to act opposite a heterology in single-strand exonuclease null mutants

Strain	Relevant Genotype	Phage	*n*	Hotspot value
SMR122	Exo$^+$	heterology	14	15 ± 0.5
SMR784	*xseA*	heterology	2	19 ± 8
SMR124	*recJ*	heterology	3	17 ± 3
SMR839	Δ*xonA*	heterology	7	23 ± 2
SMR774	*recJ xseA*	heterology	2	16 ± 0.2
SMR1262	Δ*xonA xseA*	heterology	3	16 ± 2
SMR1403	Δ*xonA recJ*	heterology	5	21 ± 2
SMR1404	Δ*xonA recJ xseA*	heterology	4	9.3 ± 0.8
SMR122	Exo$^+$	homology	11	16 ± 1
SMR784	*xseA*	homology	2	15 ± 1
SMR124	*recJ*	homology	2	17 ± 3
SMR839	Δ*xonA*	homology	5	20 ± 3
SMR774	*recJ xseA*	homology	2	15 ± 2
SMR1262	Δ*xonA xseA*	homology	3	16 ± 2
SMR1403	Δ*xonA recJ*	homology	3	25 ± 4
SMR1404	Δ*xonA recJ xseA*	homology	3	20 ± 0.9

TABLE 2-2

Ability of Chi to act opposite a heterology with the *sbcB15* allele of the gene encoding Exo I.

Strain	Relevant Genotype	Phage	*n*	Hotspot value
SMR122	Exo$^+$	heterology	27	14 ± 0.4
SMR123	*sbcB15*	heterology	20	7.7 ± 0.3
SMR775	*sbcB15 recJ xseA*	heterology	5	7.2 ± 2
SMR122	Exo$^+$	homology	16	16 ± 1
SMR123	*sbcB15*	homology	9	9.5 ± 0.4
SMR775	*sbcB15 recJ xseA*	homology	5	11 ± 1

Data and presentation are as described in Table 2-1.

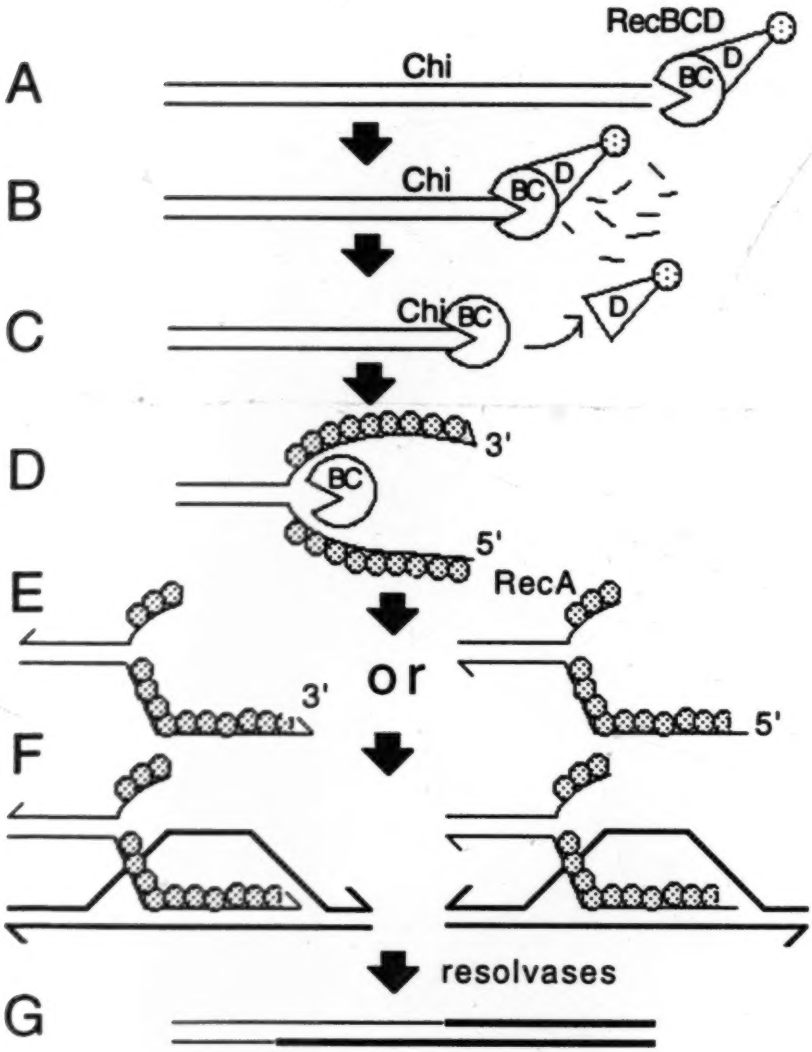


FIGURE 2-1.—A split-end model for recombination at Chi (Rosenberg and Hastings 1991). Described in text.

FIGURE 2-2. –Hotspot crosses with Chi opposite a heterology, or opposite homology. λ genetic markers, genes and mutations are as in Hendrix *et al.* (1983). The λ genotypes used follow. (A) In the crosses with Chi opposite the *imm21* heterology, the genotype of the top λ is λ *Δb1453 imm21*. *imm21* is a substitution that replaces 4.2 kb of λ DNA with ~ 3 kb of DNA that is heterologous with λ. The genotypes of the bottom phage are λ *Eam4 red(amber)270 gam(amber)210 cIts857 ChiC* (for the Chi$^+$ parent) or *cII68* (for the Chi° parent) *Sam7*. Recombinants in the Chi-stimulated interval are selected as plaques on a SuII$^+$ (*supE*) *recA* (λ *spi6 Sam7*) lysogen (SMR401) on which *Sam7* is not suppressed, *red270* and *gam210* are suppressed allowing growth on the *recA* cell, and *imm21* is selected. The unconditional *red gam* deletion *Δb1453* is counter-selected on *recA*. Recombinants in the control interval were selected on a SuII$^+$ *recD* (*imm21 Sam7*) host (SMR126), selecting for λ immunity and *S*$^+$. Alternative scoring was carried out by selecting *gam210* and *S*$^+$ on a SuII$^+$ *recA* cell (SMR125), then scoring the two intervals as turbid (Chi-stimulated interval) and clear (control interval) plaques. The *ChiC* and *cII68* alleles of the bottom parents cause clear plaque phenotype. The phage in crosses (B), with Chi opposite homology, are identical to those in crosses (A) except that the top parent has λ immunity opposite Chi. Recombinants are scored as turbid and clear plaques on a SuII$^+$ *recA* cell (SMR125) as described for crosses A. The hotspot values (Tables 2-1 and 2-2) are calculated as (turbid/clear Chi$^+$ cross) ÷ (turbid/clear Chi° cross).

In these (and all) phage λ crosses that assay RecBCD-mediated recombination, the phage must be *red gam* to inactivate the phage's Red recombination system and the Gamma protein which inactivates RecBCD enzyme. In RecABCD$^+$ *E. coli*, λ *red gam* mutants replicate *via* theta (circular monomer) mode, not rolling circle, and so must recombine in order to form a dimeric molecule as a prerequisite to packaging. Therefore, all progeny from such crosses are recombinant. These hotspot crosses, therefore, quantify Chi activity as a ratio of Chi-stimulated *vs* "base-line" recombination and do not measure frequencies of recombination. A special assay system that can measure frequencies of recombination is described in Figure 2-3. Each horizontal line represents a duplex λ DNA molecule. The diagonal lines represent regions or intervals in which recombination is selected.

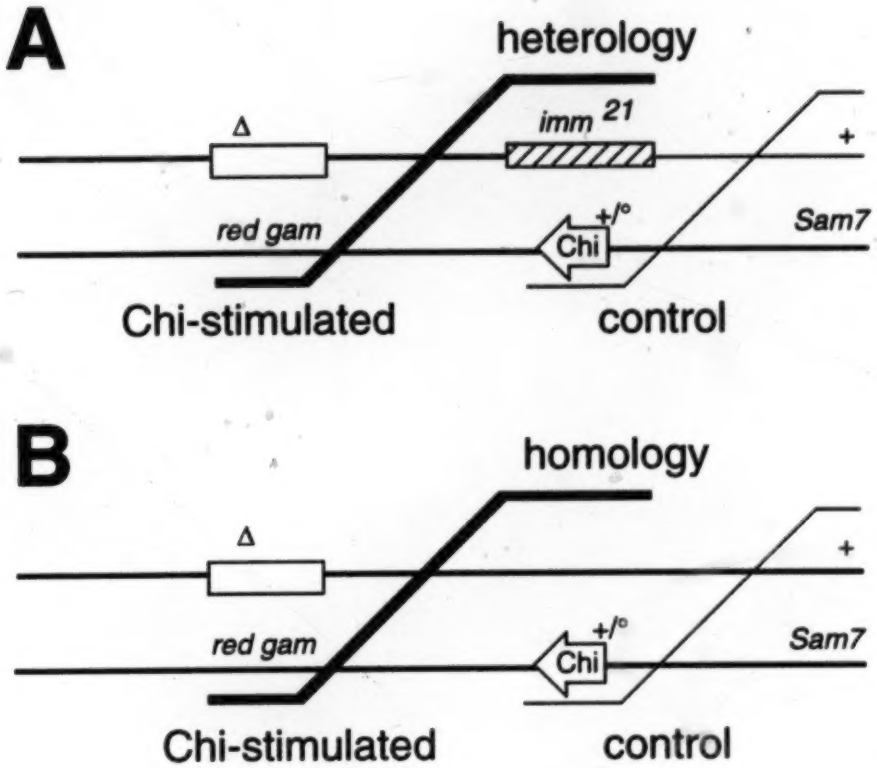
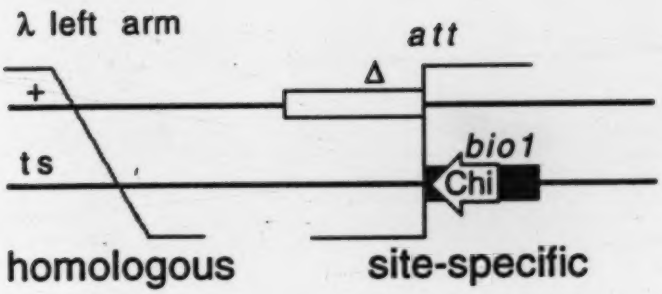

A
heterology
Δ
imm 21
+
red gam
+/°
Chi
Sam7
Chi-stimulated
control
B
homology
Δ
+
red gam
+/°
Chi
Sam7
Chi-stimulated
control

FIGURE 2-3.– Percent recombination in single-strand exonuclease defective cells. Crosses measuring recombination in the λ left arm. λ genotypes are λ *Δb516 red3 gam210* (top molecule) and λ *Mts5 Chi-bio1 Pam80* (bottom molecule). Density gradient fractions containing the site-specific recombinants were plated on a SuII$^+$ *recA* cell (SMR125) at 30° for total phage (*ts* and +) and selection for M^+ homologous recombinants from the Int-generated peak of the gradients was done by plating on a SuII$^+$ *recA* cell (SMR125) at 42°. Each bar represents the mean percent of homologous recombinants in the site-specific recombinant peak for one cross and density gradient. The black bars represent a set of crosses done in parallel and the gray bars represent a separate set. Thus this graph represents data from 16 separate crosses and phage density gradient assays. Error bars represent standard errors of the means derived from the multiple density gradient fractions assayed for total and ts^+ phage in each site-specific recombinant peak. Discussed in the text. These data were generated by S. K. Szigety.


λ left arm
att
Δ
+
ts
bio1
Chi
homologous
site-specific

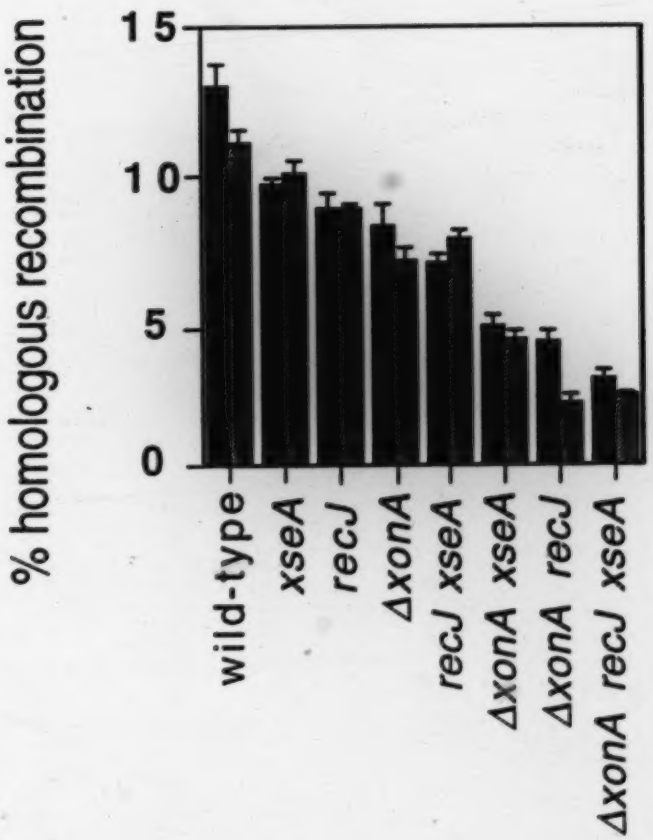
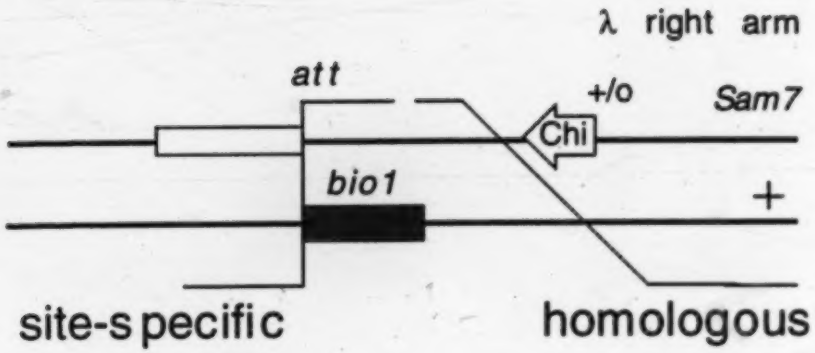
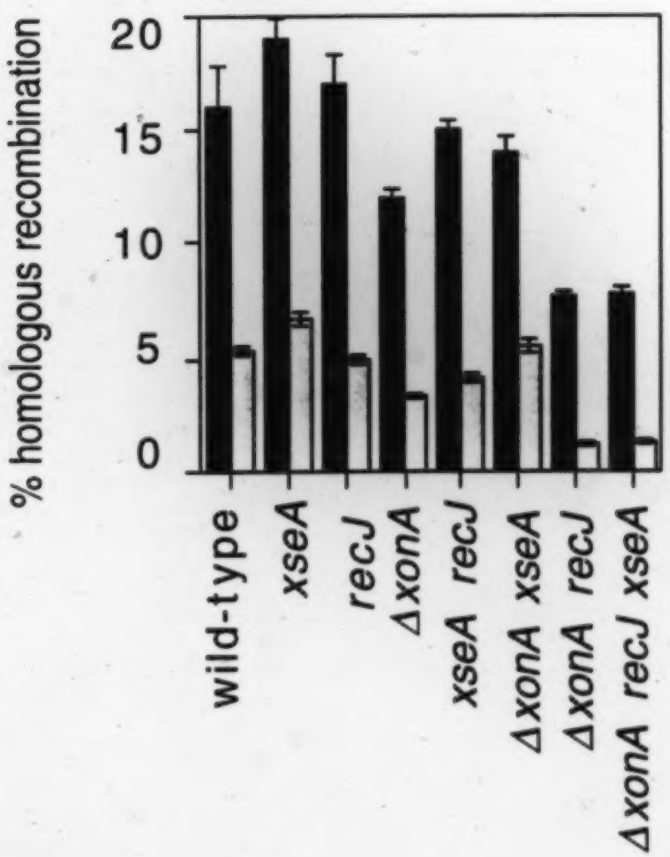

% homologous recombination
15
10
5
0
wild-type
xseA
recJ
ΔxonA
recJ xseA
ΔxonA xseA
ΔxonA recJ
ΔxonA recJ xseA

FIGURE 2-4.–Percent recombination in single-strand exonuclease defective cells. Crosses measuring recombination in the λ right arm. The genotypes of the phage used are λ *Δb527 red3 gam210 cIts857 ChiC* (Chi$^+$ parent only) *Δnin5 Sam7* (top parents) and λ *bio1 Δnin5* (bottom parent). The undeleted site-specific recombinant (densest) peak of the density gradients were plated on a SuIII$^+$ *recA* cell (SMR137) for total phage and on a SuII$^+$ *recA* cell (SMR125) for *S*$^+$ homologous recombinants. Data and presentation are as described in Figure 2-3. Dark bars represent data from the Chi$^+$ crosses. Light bars represent data from the Chi° crosses performed in parallel with the Chi$^+$ crosses. The difference between these sets illustrates Chi-stimulation of recombination. Discussed in the text. These data were generated by S. K. Szigety.


λ right arm
att
+/o
Sam7
Chi
bio1
+
site-specific
homologous


% homologous recombination
20
15
10
5
0
wild-type
xseA
recJ
ΔxonA
xseA recJ
ΔxonA xseA
ΔxonA recJ
ΔxonA recJ xseA

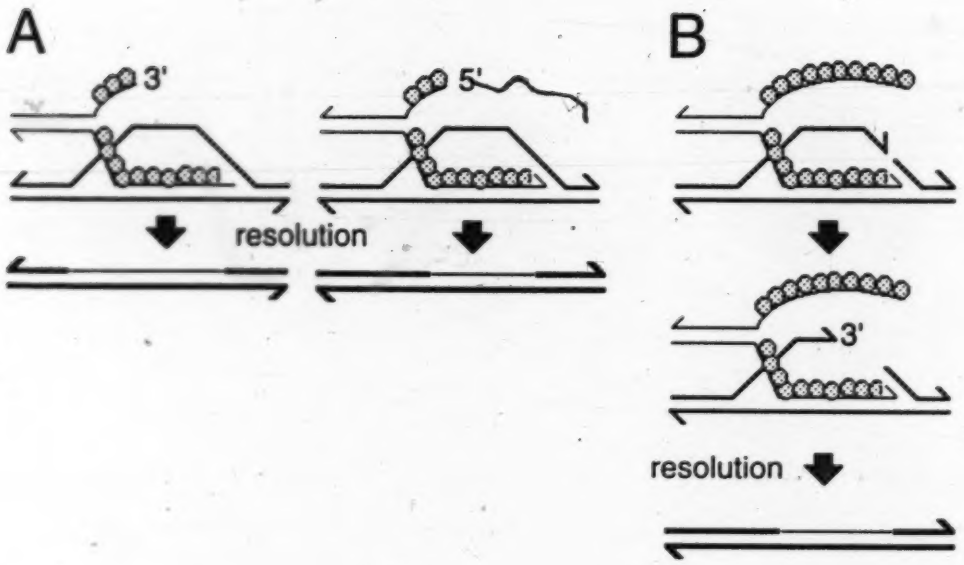


FIGURE 2-5.–Consequences of digestion of single-stranded DNA ends in the invading molecule (A) or of the invaded molecule (B). Discussed in the text. Arrow heads indicate 3' ends. Plain ends indicate 5' ends. The invading molecule is represented by thin lines and the invaded molecule by thick lines. The designations 3' and 5' indicate the ends at which an exonuclease of the designated polarities is digesting.

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3. FURTHER EVIDENCE FOR BOTH 3' AND 5' SINGLE-STRAND DNA ENDS IN RecBCD-MEDIATED RECOMBINATION.

In the split-end model for recombination at Chi (Rosenberg and Hastings 1991), RecBCD degrades DNA from a double-strand DNA end until reaching Chi. The exonuclease activity is then converted, by loss or inactivation of the RecD subunit, into a helicase activity which produces single-stranded 3' and 5' ends, both of which are proposed to be recombinagenic.

In the previous chapter, evidence was presented supporting the idea that both 5' and 3' ends exist in recombination intermediates. I found that the presence of single-strand exonucleases of either 5' and 3' polarity is necessary for full Chi activity when Chi acts opposite heterologous DNA. We also found that the presence of single-strand exonucleases of either 5' or 3' polarity is necessary for full levels of RecBCD-mediated recombination (data kindly provided by S. K. Szigety). In this chapter, I present additional evidence that single-strand DNA ends exist in intermediates in Chi-stimulated recombination

First, transductional recombination experiments were performed to generalize the finding that the absence of single-strand-dependent exonucleases (RecJ, ExonucleaseI, Exonuclease VII) decreases recombination frequency (Chapter 2). Second, I performed experiments to assess whether the absence of single-strand-dependent exonucleases alters the distribution of Chi-stimulated recombination events along a lambda chromosome. If the absence of single-strand exonucleases changes the distribution of Chi-stimulated recombination, this would provide

independent support for the idea that both 3' and 5' single-strand ends exist in intermediates during recombination.

The distribution of RecBCD-mediated recombination events along a lambda chromosome was determined by McMilin *et al.* (1974). They found the distribution of exchanges along the chromosome to be fairly uniform. Upon the introduction of a Chi site, a ten fold increase in the titer of recombinants was observed at the Chi site, and extending to the left of Chi (on the standard λ map) decreasing gradually over a long distance. A possible reason for the gradual decrease leftward is that single-strand-dependent exonucleases, Exonuclease I, RecJ, and Exonuclease VII, might move the site of strand invasion leftward along λ by trimming the single-strands ends away from Chi (Figure 3-1). If this were so, then in the absence of exonucleases, the gradual leftward slope of Chi-stimulated recombinants might disappear and a steeper leftward slope might exist because more of the strand invasion events would occur right at Chi. This result, if obtained, would provide further evidence that the substrates for these nucleases (single-stranded 3' and 5' ends) exist during an intermediate step in recombination *in vivo*. Crosses were carried out to detect whether a steeper slope of exchanges leftward of Chi occurs in exonuclease-deficient cells.

MATERIALS AND METHODS

Bacteria and phage: All of the bacterial strains used as cross hosts in this work are Su^- (see Table 3-1). SMR122 is Exo^+. SMR123 and SMR124 carry the *sbcB15* (Kushner *et al.* 1971) and *recJ284*::Tn*10* (Lovett and Clark 1985)

mutations respectively. Δ*xonA300*::*Cat* (Chapter 2, and Appendix B) and Δ(*xseA-gua*)*zff3139*::Tn*10kan* (Vales *et al.* 1979; obtained from Dr. Susan Lovett, Brandeis University) were used. Strains carrying combinations of the exonuclease-deficient alleles were constructed by standard P1 transduction methods. P1 donor strains used in transductional crosses carried the alleles *recQ61*::Tn3 (SMR279) and *zai2006*::Tn3 (594 *zai2006*::Tn3; see Table 3-1). The phage lambda stocks used were grown from stocks in the laboratory collection and are designated λHR11 (λ *bio1* Δ*nin5*), λHR19 (λ Δ*b2 red3 gam210 cI857 Rts129*), and λHR70 (λ Δ*b2 red3 gam210 cI857 Chi*$^+$*D Rts129*) (see Appendix A).

P1 transduction assay: Bacteriophage P1(*vir*) was grown on Tn3-containing bacteria carrying either *zai2006*::Tn3 or *recQ61*::Tn3. 0.2 ml of recipient saturated cultures grown in LBH broth (Luria-Bertani-Hersokowitz; NaCl, yeast extract, tryptone, pH 7.5) were infected with approximately $1x10^7$ P1 particles added to 0.5ml of 15mM $CaCl_2$ 30mM $MgSO_4$ buffer (MC buffer). LBH broth was added to a separate aliquot of cells (in MC buffer) and was used as a phage-free control. The mixtures were incubated (shaking) for 20 minutes at 37°, pelleted and resupended in 0.5 ml LBH broth containing 20mM sodium citrate. Sodium citrate prevents further adsorption of P1 particles to cells by removing calcium ions which are required for the adsorption process (Miller, 1977).

After shaking for 60 minutes at 37° (to express ampicillin resistance), the transduction mixtures were spread onto LBH ampicillin (100μg/ml) plates containing 20mM sodium citrate and incubated for approximately 24 hours. 0.1ml of the P1 stock was also plated on LBH ampicillin plates to

test that bacterial contamination of the stock did not give rise to any drug resistant colonies. On the same day as the transduction experiment, titers of P1 were determined on all the recipients to ascertain that P1 infected all the strains equally well, and this was observed. Viable cell concentrations of the Exo^+ and Exo^- recipient cultures were determined by diluting and plating on LBH plates.

To calculate the frequency of transduction, the number of ampicillin-resistant (AmpR) colonies counted for each genotype was divided by the number of P1 particles added for each genotype. To ensure that all P1 particles infected cells, the ratio of phage to cells was at least 1:15.

Crosses to measure the distribution of recombination left of Chi in Chi-proximal and Chi-distal intervals: *bio1*-carrying phage (λHR11, Appendix A, see Figure 3-5) were crossed both to a $Chi^+\lambda$ and to a $Chi^\circ\lambda$ (λHR70 and λHR19, Appendix A, see Figure 3-5) separately in each strain. Each phage was added at a multiplicity of approximately 7-13 per cell. Lambda crosses were performed as follows: 0.1ml of each saturated bacterial culture to be assayed was inoculated into 10ml mating culture broth (MCB) [tryptone broth containing 1M MgSO4, 2mg/ml thymine and 5mg/ml vitamin B1]. The cultures were grown shaking at 37° to a concentration of approximately $1.5x10^8$ cells per ml. Phage stocks were assayed the day before to determine titers, and approximately $5x10^8$ phage of each genotype were added to 0.5 ml of cross host.

After a period of adsorption at 37°, shaking for 30 minutes, iced TM (10mM TRIS $MgSO_4$, pH 7.4) buffer was added, the cross mixture was pelleted, and the supernatant was decanted to remove most of the

unadsorbed phage. Cells were resuspended in 5ml of MCB at 37°. One ml was extracted, chloroformed and titered on permissive host, SMR130, to determine the number of remaining unadsorbed phage. Cross mixtures were bubbled in a waterbath at 37° for one hour. Chicken egg white lysozyme (Sigma) and chloroform was added to cross mixtures to lyse and kill the cells, releasing progeny phage. Cross lysates were assayed on SuII *recD* (SMR130) at 32° to determine the total phage yields (i.e. parents, recombinants, and any residual unadsorbed phage). Recombinants were assayed on SuII *recA* (SMR125) at 42°. SuII *recA* selects against the *bio1* substitution which is unconditionally *red*$^-$*gam*$^-$. *gamam210* is selected because this amber *gam* allele is suppressed in SuII cells. R^+ is selected by plating at 42°. In the crosses involving the Chi$^+$ phage, recombinants that formed as a result of recombination events rightward of the c*Its*857 marker give clear plaques (c*Its*857 confers clear plaque phenotype at 42°). Recombinants that formed as a result of recombination events leftward of the *cIts857* marker (but still in the Chi-stimulated region) give turbid plaques. Similar crosses were carried out but using a Chi° control phage. The theta replication of *red*$^-$*gam*$^-$λ in the Su$^-$ Rec$^+$ cross hosts produces circular monomers which must dimerize *via* recombination in order to be packaged. Thus all the progeny phage have recombined. Therefore a distribution, not a frequency, of recombination events is measured. To measure the distribution of exchanges leftward of Chi, the ratio of (interval II recombinants [clears] /interval I recombinants [turbids] in a Chi$^+$ cross) ÷ (interval II recombinants/interval I recombinants in a Chi° cross) was used.

RESULTS AND DISCUSSION

Both 5' and 3' single-strand dependent exonucleases are required for efficient transductional recombination: We found previously that the presence of single-strand-dependent exonucleases of either 5' or 3' polarity is necessary for full levels of RecBCD-mediated recombination in crosses of *red⁻ gam⁻* λ phages (Figures 2-3 and 2-4, data kindly provided by S. K. Szigety). The absence of single-strand exonucleases of both polarities decreased overall recombination frequency about four-fold, indicating that the substrates for these exonucleases, 3' and 5' single-strand ends, exist in some intermediate in recombination (Chapter 2).

Is this finding specific to λ by λ RecBCD-mediated recombination? Results of Meisel and Roth (1996) with two of the three single-strand exonuclease-deficiencies in Salmonella recombination suggest that the partial requirement for single-strand exonucleases may be general. To generalize the above observations in *E. coli*, I tested whether absence of single-strand-dependent exonucleases of both 3' and 5' polarities decreases the amount of RecBCD-mediated recombination in a phage P1-mediated transductional assay. P1 transductional crosses were performed in strains proficient or deficient for the three single-strand exonucleases RecJ, ExoI and ExoVII. P1 lysates were grown on strains carrying ampicillin-resistance markers (Tn3) and were used to transduce recipient strains to ampicillin resistance. The frequencies of transductional recombination were determined as the number of AmpR transductants obtained from each cross divided by the number of infectious P1 particles added in crosses in which recipient cells were added in excess.

When *recQ61*::Tn3 was the transduced marker, the frequency of transductional recombination in a Δ*xonA recJ* Δ*xseA* strain was approximately four-fold lower than the isogenic Exo$^+$ control (Figure 3-2; data in Appendix C1a). This is similar to what was reported for λ by λ RecBC-mediated recombination in Chapter 2. In Δ*xonA recJ* cells, the frequency of recombination was slightly higher than Δ*xonA recJ* Δ*xseA* (Figure 3-2). The frequency (about 3-fold lower than Exo$^+$) is similar to previous results with λ. A somewhat surprising result, which appears to be specific to the *recQ61*::Tn3 transductional experiments, is that in *recJ* Δ*xseA* cells, the recombination frequencies were similar to the triple mutant strain (Figure 3-2). A possible explanation may be that certain sequences around the chromosome may dictate that specific strand polarity dominate a specific region. For example, the DNA around *recQ* may be insensitive to ExoI because the sequence may contain ExoI pause sites (hypothesized here). Removal of RecJ and ExoVII might be sufficient to remove all 3' and 5' single-strands capable of acting at this region.

P1 transductional recombination frequencies assayed using the *zai2006*::Tn3 marker are shown in Figure 3-3. Δ*xonA recJ* Δ*xseA* cells had a five-fold lower recombination frequency than Exo$^+$. Δ*xonA recJ* cells had approximately a three-fold lower recombination frequency than Exo$^+$ (Figure 3-3). The extreme drop in recombination frequency in *recJ* Δ*xseA* cells seen at *recQ* is not observed with *zai2006*::Tn3.

In conclusion, in λ by λ crosses (Figures 2-3 and 2-4), and in both sets of transduction experiments, triple exonuclease-deficient cells show reduced recombination. Therefore the observation that the absence of single-strand-specific exonucleases reduces the frequency of recombination

appears to be a general feature of RecBCD-mediated recombination, and is not specific to the λ assay.

Single-strand-specific exonucleases could aid in recombination by degrading one or the other strand in a split-end intermediate. This would ensure that the DNA would remain single-stranded, and so recombinagenic, even if it re-annealed after RecBC helicase passed (Chapter 2; Miesel and Roth 1996).

Both 5' and 3' single-strand-dependent exonucleases alter the distribution of Chi-stimulated exchanges: The evidence that both 3' and 5' ends occur at Chi sites *in vivo* (Razavy *et al.* 1996; Chapter 2) suggested a possible explanation for an old observation about Chi-stimulated recombination: that Chi-stimulated recombination decreases gradually over a long distance downstream (left) of Chi (McMilin *et al.* 1974). A graph of this frequency of recombination *vs.* distance is shown in Figure 3-4.

One hypothesis for why this leftward decline of Chi-stimulated exchanges exists could be that exonucleases, RecJ, ExoVII and ExoI, could move the site of strand exchange leftward by trimming the strand that will invade (Figure 3-1). If true, then in the absence of the single-strand exonucleases, a sharp peak might be observed at Chi with no gradual leftward decline. To compare the steepness of slopes of exchanges left of Chi in Exo$^+$ and Δ*xonA recJ* Δ*xseA* cells, crosses measuring Chi-stimulated recombination in two intervals left of Chi, one proximal to Chi and one distal to Chi--but within the Chi-stimulated region, were done (Figure 3-5). Because the distal interval is 4.5 to 12 kb away from Chi$^+$D, and Chi

stimulation has been detected as far away as 10 kb, (Lam *et al.* 1974; McMilin *et al.* 1974), Chi-stimulated exchanges can occur in the distal interval.

By taking a ratio of the events close to and farther from Chi, to Chi's left, (in both Chi$^+$ and Chi$^\circ$ crosses), one obtains a value that provides an indication of how steep the leftward slope of exchanges is (see Figure 3-5). These crosses do not measure frequencies of recombination, but rather the distribution of recombination events along the lambda chromosome. Results in Table 3-2 show no apparent difference in the ratio of leftward proximal to distal exchanges, measured as recombination in (Proximal/Distal Chi$^+$) ÷ (Proximal/Distal Chi$^\circ$), between Exo$^+$ and the triple mutant strain. Two different mutations in the Exonuclease I gene, Δ*xonA* (null) and *sbcB15* (partial function), were tested. The Chi slope values obtained for both alleles do not differ significantly from Exo$^+$ (Table 3-2).

Interestingly, the clear (proximal)/turbid (distal) values in both the Chi$^+$ and Chi$^\circ$ crosses show a two-fold increase in the Δ*xonA recJ* Δ*xseA* strain compared with Exo$^+$. These results indicate that, regardless of the presence of Chi, absence of the single-strand dependent exonucleases produces more recombination rightward. If the presence of Chi is required for single strand ends to be formed (through RecBCD action), how could the absence of these nucleases produce any effect in a Chi$^\circ$ cross? One possibility could be that the Chi-like sequence (which differs from Chi$^+$ by a single base pair, and which is naturally present at each λ site that can mutate to Chi$^+$), could promote partial Chi activity in the Chi$^\circ$ cross (Cheng and Smith 1984). Partial Chi activity of Chi-like sequences has

been documented (Cheng and Smith 1984). This could account for the residual recombination seen in the Chi° crosses and also for the effect the absence of exonucleases have on the interval ratios.

The proximal to distal ratio of exchanges in Chi⁺ and in Chi° crosses, provides support for the idea that action of single-strand exonucleases influences the distribution of exchanges along the DNA molecule recombining. To test more specifically whether the leftward decline is affected, density profile experiments should be carried out. Density profiles show the distribution of recombination along the lambda chromsome as depicted in Figure 3-4. The hotspot effects of Chi and the gradual leftward decline were first detected in such experiments (Stahl *et al.* 1975) (Figure 3-4).

TABLE 3-1 *E. coli* K12 strains

SMR number	Strain	Relevant genotype	Source or Reference
122	JC11450[1]	Su- *rec+*	A.J. Clark
123	JC11451	Su- *sbcB15*	A.J. Clark
124	FS2504	JC11450 *recJ284*::Tn*10*	F. W. Stahl
125	AFT196	C600[2](SuII) Δ*(srlR-recA)306*::Tn*10*	A. Taylor
130	FS1576	C600 (SuII) *recD1009*	Stahl *et al.* (1986)
264	AC417	594[3] (Su^-) *recD1014*	A. Chaudhury
279	RDK1647	KD1996[4] *recQ61*::Tn*3*	Nakayama *et al.* (1985)
774	SZ774	AB1157 Su^- Δ*(xseA-gua) zff3139*::Tn*10kan recJ284*::Tn*10*	Razavy *et al.* (1996)
784	SZ784	AB1157 Su^- Δ*(xseA-gua) zff3139*::Tn*10kan*	Razavy *et al.* (1996)
839	HR839	JC11450 Δ*xonA300*::*Cat*	Razavy *et al.* (1996)
1262	HR1262	AB1157 Su^- Δ*(xseA-gua) zff3139*::Tn*10kan* Δ*xonA300*::*Cat*	Razavy *et al.* (1996)
1403	SZ1403	JC11450 Δ*xonA300*::*Cat recJ284*::Tn*10*	Razavy *et al.* (1996)
1404	SZ1404	AB1157 Su^- Δ*xonA300*::*Cat recJ284*::Tn*10* Δ*(xseA-gua) zff3139*::Tn*10kan*	Razavy *et al.* (1996)
3440	EG312	Δ*(gpt-lac) tyrT*$^-$ F'*lacZ813 lacI22 zai2006*::Tn*3*	*E.coli* Genetic Stock Centre
—	—	594 *zai2006*::Tn*3*[5]	Harold Bull

[1]JC11450 is a Su^- derivative of AB1157 (also carries the following markers: *thr1 leuB6 thi1 proA his4 argE3 lacY1 galK2 ara14 xyl15 rpsL31 tsx33* Δ*rac*)
[2]C600 background carries the following markers: *thi thr leu* (Appleyard, 1954)
[3]594 background carries the following markers: *SmR gal1 gal2* (Weigle, 1966)
[4]KD1996 background carries the following markers: *polA12 rpsl ton ilv45 trpC3 pro mtl1 malA ara9 galK2 lacI14* (Nakayama *et al.* 1985)
[5]Strain was not saved in SMR collection (*zai2006*::Tn*3* derived from SMR 3440).

TABLE 3-2
Ratio of leftward exchanges proximal and distal to Chi in exonuclease-deficient mutants.

Relevant Genotype	phage	cross 1 clears/turbids at 42°	cross 2 clears/turbids at 42°	Average	± var	Proximal / distal value.
Exo⁺	Chi⁺	2.589	2.785	2.687	0.098	3.4
	Chi°	0.7833	0.7966	0.7900	0.0067	
sbcB15	Chi⁺	2.381	3.033	2.707	0.33	4.0
	Chi°	0.6775	0.6854	0.6815	0.0040	
ΔxonA	Chi⁺	3.212	3.058	3.135	0.077	4.3
	Chi°	0.6863	0.7688	0.7276	0.041	
ΔxseAΔxonA recJ	Chi⁺	5.331	5.600	5.466	0.13	4.0
	Chi°	1.458	1.290	1.374	0.084	

Crosses diagrammed in Figure 3-5 were performed in the strains indicated above (see Table 3-1 for names and full genotypes of these strains). A ratio of recombinants occuring proximal/distal to Chi, to Chi's left is given for each cross (see Figure 3-5). A Chi° control cross is also performed for each strain in parallel. This proximal/distal value is the ratio of clear plaques/turbid plaques when Chi is present over the ratio of clear plaques/turbid plaques when Chi is absent. The value gives an indication of how steep the slope of exchanges leftward from Chi is. The "± var" values are calculated as for standard deviations but with data from 2 independent experiments. 130-1600 plaques of each morphology (clears and turbids) were scored to generate the data for each cross.

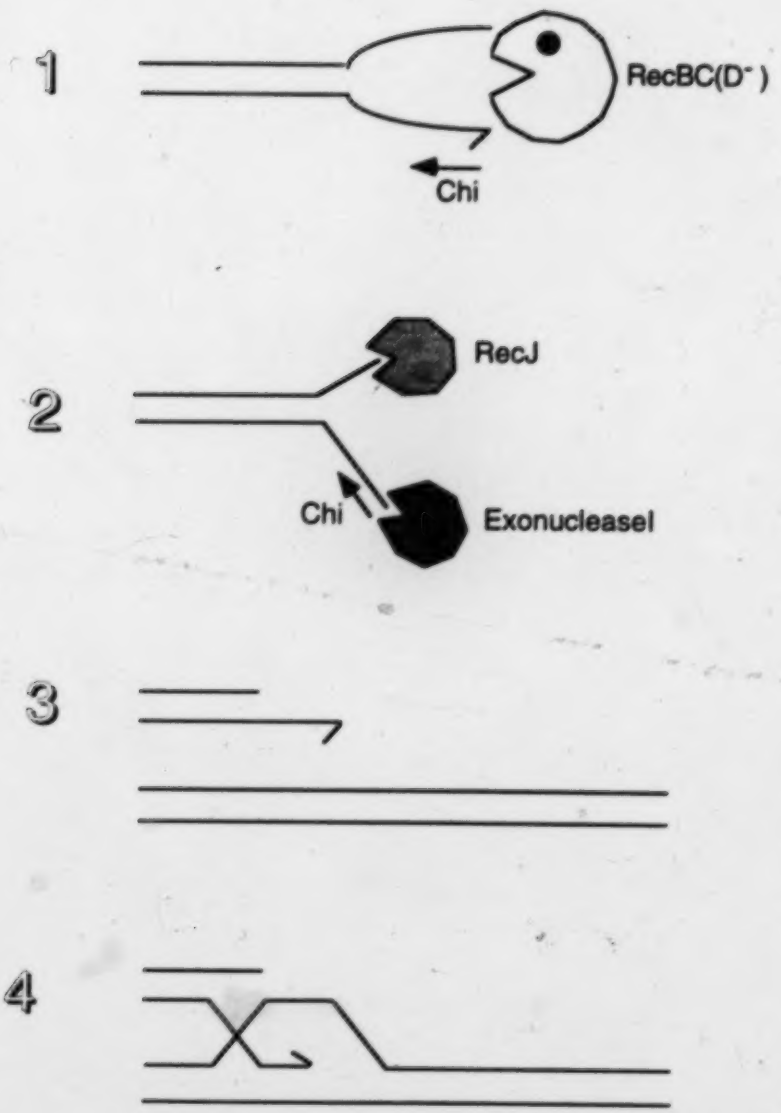
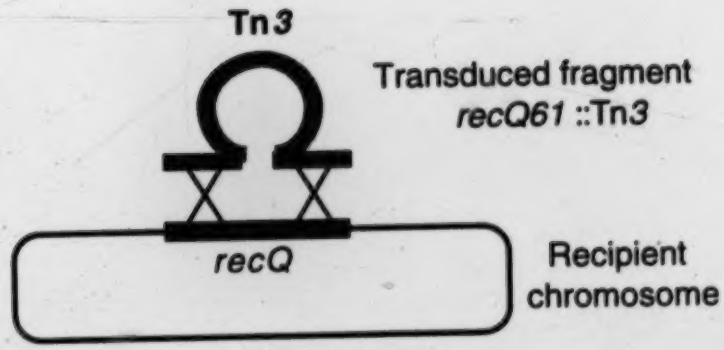


FIGURE 3-1.— Single-strand-dependent exonucleases ExoI, RecJ, or ExoVII (not shown) could move the site of strand invasion leftward along λ by trimming the single-strand ends away from Chi.

FIGURE 3-2.— Frequencies of transductional recombination using a *recQ61*::Tn3 P1 donor. Frequency measurements presented in graph are all normalized to wild-type (which is set to 1.00). The actual frequency of the wild-type strain is $5.3 \times 10^{-5} \pm 1.4 \times 10^{-6}$. The error bars represent one standard error of the mean. The data are from 3 independent experiments performed as described (Materials and Methods) except for *recJ ΔxseA* --only two experiments were performed. The *recQ61*::Tn3 P1 donor cell is SMR279. Note: Because the transductional recombination events assayed here use pre-existing recombination proteins in the recipient cells, using *recQ61*::Tn3 as an ampicillin-resistant marker should not affect recombination. The cross hosts used are (from left to right on graph) SMR122, SMR839, SMR124, SMR784, SMR774, SMR1262, SMR1403, SMR1404. For the number of Amp^R transductants scored per transduction, refer to Appendix C-1 (a).


Tn3
Transduced fragment
recQ61 ::Tn3
recQ
Recipient
chromosome

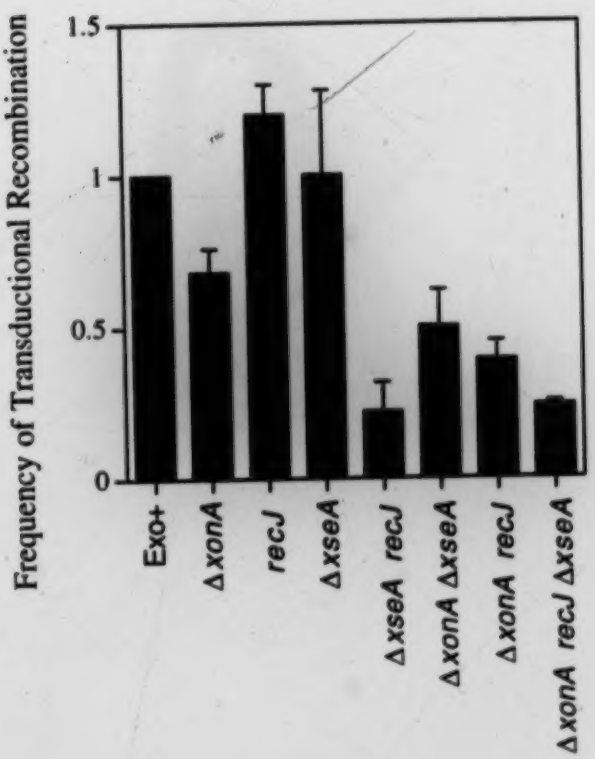
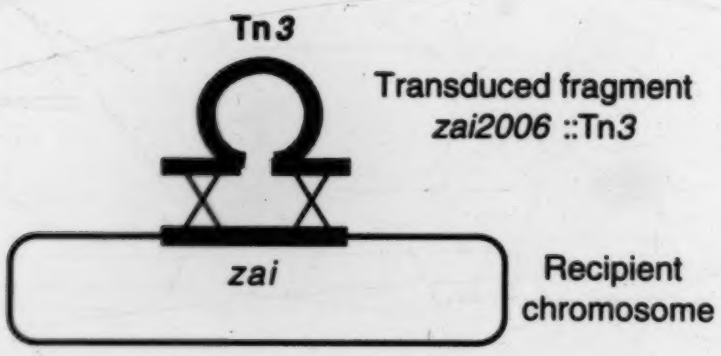
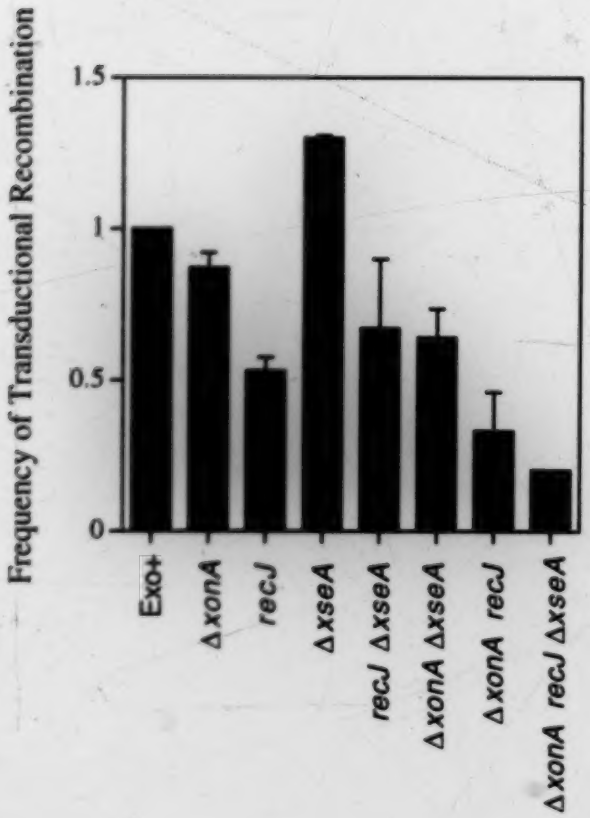

Frequency of Transductional Recombination
1.5
1
0.5
0
Exo+
ΔxonA
recJ
ΔxseA
ΔxseA recJ
ΔxonA ΔxseA
ΔxonA recJ
ΔxonA recJ ΔxseA

FIGURE 3-3.— Frequencies of transductional recombination using a *zai2006*::Tn3 P1 donor. Frequency measurements presented in graph are all normalized to wild-type (which is set to 1.00). The actual frequency of the wild-type strain is $7.9 \times 10^{-6} \pm 2.5 \times 10^{-6}$ (SD). The error bars are calculated as for standard deviations but with data from 2 independent experiments (Materials and Methods). The *zai2006*::Tn3 P1 donor cell is 594 *zai2006*::Tn3 (see Table 3-1) which was constructed by transducing *zai2006*::Tn3 from SMR3440 into 594. The cross hosts used are (from left to right on graph) SMR122, SMR839, SMR124, SMR784, SMR774, SMR1262, SMR1403, SMR1404. For the number of Amp^R transductants scored per transduction, refer to Appendix C-1(b).


Tn3
Transduced fragment
zai2006 ::Tn3
zai
Recipient
chromosome


Frequency of Transductional Recombination
1.5
1
0.5
0
Exo+
ΔxonA
recJ
ΔxseA
recJ ΔxseA
ΔxonA ΔxseA
ΔxonA recJ
ΔxonA recJ ΔxseA

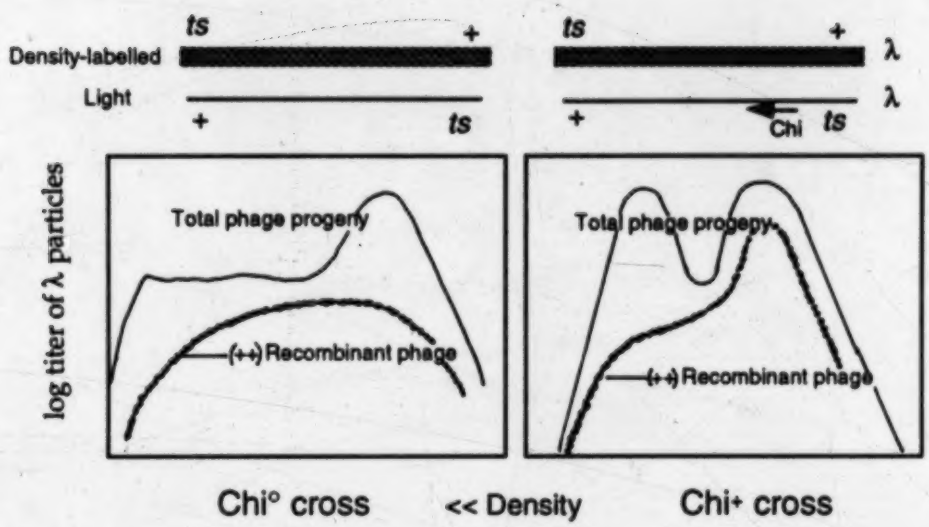


FIGURE 3-4. — A density profile experiment (Stahl *et al.*1975). One parental phage chromosome is density labeled with [^{13}C] glucose and $^{15}NH_4Cl$ (thick line). The other parent is not (thin line). The phage are allowed to recombine. The ++ recombinants range from being nearly fully heavy to nearly fully light depending on where the recombination event takes place. Both phage carry *Pam80*, a replication gene amber allele, and the host is *dnaBts* allowing a "double-block" for replication. Cross lysates are centrifuged to equilibrium in cesium formate and fractions are collected. Earlier fractions contain denser phage; later fractions contain less dense phage. Fractions are titered at permissive temperature (to see the parental peaks) and at restrictive temperature at which only ++ recombinants form plaques. A uniform distribution of recombination events along the chromosome (see left picture) is seen when Chi is absent. However, if Chi is present, a peak will be obtained among the recombinant titers in the vicinity of the λ genome where Chi is (see right picture). The peak gradually decreases to the left on the λ map (leftward decline). The graphs are tracings of data published by Stahl *et al.* (1975).

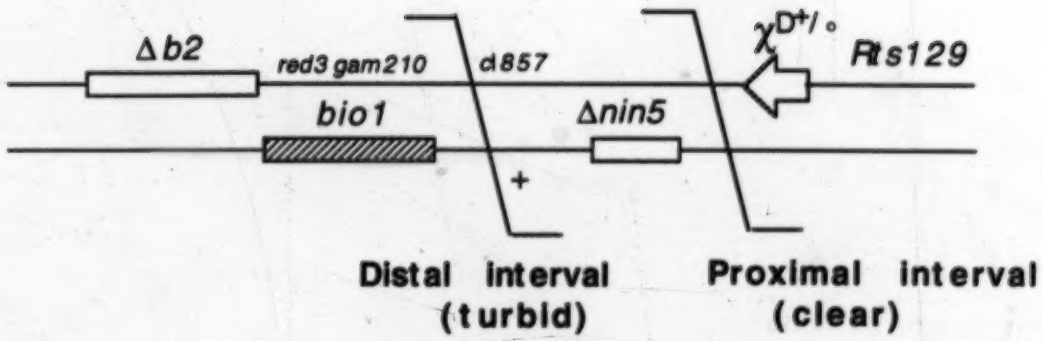


FIGURE 3-5.— Crosses used to determine distribution of exchanges distal and proximal to Chi, in the Chi-stimulated region left of Chi. Recombination events in both intervals are stimulated by Chi. Because the distal interval begins 4.5 kb away from Chi+D, and Chi stimulation has been detected as far away as 10 kb, most exchanges in the distal interval are probably Chi-stimulated (Lam *et al.* 1974; McMilin *et al.* 1974). The proximal interval is stimulated to a greater extent than the distal interval because it is closer to Chi. Each horizontal line represents a duplex lambda DNA molecule and the diagonal lines represent intervals in which the recombination events are selected.

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4. THE EFFECT OF INTERVAL SIZE ON RECOMBINATION IN THE ABSENCE OF SINGLE-STRAND-SPECIFIC EXONUCLEASES

Using Salmonella transductional recombination assays in Exo$^+$ and RecJ$^-$ ExoI$^-$ cells, Miesel and Roth (1996) found that RecBCD recombination works less well in the absence than in the presence of the single-strand-dependent exonucleases. We obtained similar results with RecJ$^-$ ExoI$^-$ ExoVII$^-$ *E. coli* in P1 transductional assays (Chapter 3; Figures 3-2 and 3-3), and using phage λ *red*$^-$ *gam*$^-$ (Chapter 2; Figures 2-3 and 2-4). For Meisel and Roth (1996), the effects of single-strand dependent exonuclease mutations on RecBCD-mediated recombination appeared to be more pronounced when the interval in which recombination events occur was very short.

In crosses measuring the amount of Chi-activity when Chi is opposite a heterology (depicted in Figure 4-1A), the ratio of recombinants in the Chi-stimulated interval (left of Chi) over recombinants in the control interval (right of Chi), in both Chi$^+$ and Chi$^\circ$ crosses, was lower in strains lacking RecJ and ExoI than in Exo$^+$ cells (Table 4-1, a more detailed presentation of the data summarized in Tables 2-1 and 2-2). The absence of ExoVII contributed slightly to this effect.

Two possibilities exist as to why left / right interval ratios are reduced in exonuclease-deficient cells as compared with Exo$^+$. First, the recombination in the right interval could be increased in Exo$^-$ cells relative to the same interval in Exo$^+$ cells. Alternatively, recombination in the left interval could be decreased in Exo$^-$ strains relative to Exo$^+$ cells. The latter possibility appears probable for two reasons: first, because the overall frequency of recombination in Exo$^-$ cells is decreased relative to

Exo$^+$ (Figure 2-3, Figure 2-4, Figure 3-2, Figure 3-3); and second, because the left interval (1.5 kb) is approximately five times shorter than the right interval (6.7 kb). Following the Miesel and Roth idea (1996), recombination would be reduced in small intervals when there are no single-strand exonucleases present.

To address this hypothesis, we carried out heterology hotspot crosses using phage in which the size of the right interval is reduced dramatically, to between 69-965 basepairs, making it less than or about equal to the left interval (see Figure 4-1C). Because the left and right intervals are much closer in size, the Miesel and Roth (1996) hypothesis predicts decreased recombination in the right interval, and a consequent increase in the left/right interval ratio in the Exo$^-$ mutants.

MATERIALS AND METHODS

Bacteria and phage: All of the bacterial strains used as cross hosts have been described earlier (Chapter 2; Materials and Methods, Chapter 3; Table 3-1). SMR122 (JC11450) is a Su$^-$ derivative of AB1157 (Rec$^+$) and serves as our control strain. SMR123 (JC11451) is Su$^-$ *sbcB15*, and *sbcB15 recJ284*::Tn*10* (SMR729) was constructed from SMR123. Derivatives of JC11450 are *recJ284*::Tn*10* (SMR124), Δ*xonA300*::*Cat* (SMR839), and Δ*xonA300*::*Cat recJ284*::Tn*10* (SMR1403). A spontaneous Su$^-$ derivative of the AB1157-derived strain *sbcB15 recJ284*::Tn*10* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (STL1607; obtained from S. Lovett, Brandeis University) was isolated by S. Szigety (designated as SMR775). Δ*xonA300*::*Cat recJ284*::Tn*10* Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (SMR1404) was constructed

using another spontaneous Su⁻ derivative of the AB1157 strain Δ(*xseA-gua*) *zff3139*::Tn*10Kan* (STL1605). This Su⁻ derivative is designated as SMR784; see also Table 6-1 for more information on SMR784 and SMR775).

λ phages used for standard heterology and homology crosses are described in Chapter 2 (Figure legend 2-2) and are from the laboratory collection. λ*redam270 gamam210 Chi*⁺*C Ots28* (λHR89), and λ*redam270 gamam210 Chi*° *Ots28* (λHR90) were constructed as follows: a λ phage with the genotype Δ*b1453 imm21 Pam80* was crossed with λdv [pSR1] in SMR1992. λdv is a plasmid containing a part of λ including *ori*, and the *O* and *P* replication genes. The one used here also carries a kanamycin resistance marker (Matsubara and Kaiser 1969; Rosenberg 1988). The λdv used for the cross carried a temperature sensitive mutation in the *O* gene, *Ots28*. Crosses were performed in a Su⁻ cross host (SMR1992) at 32°C and *P*+ recombinants were selected on Su⁻ *recD* strain (SMR264) at 30°C. *Ots28*-containing recombinants were screened by toothpicking *P*+ recombinant plaques onto the Su⁻ *recD* bacterial lawn and incubating at 30°C and 42°C. Five *ts* candidates were purified and retested (absence of plaque formation at 42°C). The *ts* phage were grown up at 32°C. The λΔ*b1453 imm21 Ots28* candidates were genotype tested: Δ*b1453* (unconditional *red*⁻ *gam*⁻) was confirmed by the ability to form plaques on a SuII (P2) lysogen and the inability to form plaques on a SuIII *recA* host (Hendrix *et al.* 1983). *imm21* was confirmed by the failure to form plaques on a λ*imm21* lysogen, and *Ots28* was confirmed by the inability to form plaques on a permissive host at 42°C. In a λdv by λ cross, one can also obtain splice recombinants in which the entire λdv is incorporated into

the λ chromosome creating a longer molecule (e.g. Rosenberg 1988). To screen the five candidates for patches, each candidate was mixed with equal concentrations of the normal-sized parental phage and mixtures were centrifuged to equilibrium in cesium formate density gradients. The bottom of each centrifuge tube was punctured with a needle, and 2-drop fractions were collected and assayed on hosts, SMR264 and SMR130, that select either the candidate or the control (normal-sized λ parent). The parental phage, λ *Δb1453 imm21 Pam80*, can grow on SMR130 (SuII *recD*) at 42°C whereas the candidate, λ *Δb1453 imm21 Ots28*, cannot. The candidate grows on SMR264 (Su^-) at 30°C whereas the parent cannot. Phage in earlier drops are denser (phage have longer DNA molecules); phage in later drops are less dense (phage have normal length DNA molecules) (Rosenberg 1988). The cesium formate density gradient results showed that the candidates tested were of the same density as the normal-sized λ parent because both appeared later in the gradient and the peaks obtained were superimposable. The λ*P*$^+$ *Ots28* recombinants do not have λdv incorporated into their genomes; the recombinants appear to be patches*. In the last step in the construction of λ *red gamam210 Chi*$^+$ *Ots28* phage and the *Chi*° derivative, λ*Δb1453 imm21 Ots28* phages were crossed with λ*Eam4 red270 gamam210 cI857 Chi*$^+$C Sam7 (λSR4) and λ*Eam4 red270 gamam210 cII68 Sam7* (λSR5). λ*red270 gamam210 S*$^+$ recombinants from both crosses were selected on a SuII *recA* host, SMR125, at 30°C. *Ots28* was screened as plaques that do not survive at 42°C. The frequencies of reversion of the *Ots28* marker carried by the λ *red gamam210 Chi*$^+$/° *Ots28* candidates were determined to be about 10^{-5}. Absence of *imm21* in these

* Isolates were not saved in the λHR collection.

phages was confirmed by observing growth on an *imm21* lysogen but not on an *immλ* lysogen (Hendrix *et al.* 1983). The *Chi*$^+$ and *Chi*$^\circ$ status of the phages were checked by plating on a Su$^-$ *rec*$^+$ (SMR128) host and observing larger plaques with the Chi$^+$ phage and smaller plaques with the Chi$^\circ$ phage (Stahl *et al.* 1975). A single stock of each λ*red270 gamam210 ChiC*$^+$ *Ots28* and λ*red270 gamam210 Chi*$^\circ$ *Ots28* was chosen and designated λHR89 (λSR453) and λHR90 (λSR454) respectively.

Heterology and homology hotspot crosses: Crosses were carried out as described (Chapter 3, Materials and Methods; Stahl *et al.* 1983). Total cross progeny and unadsorbed phage were assayed on SuIII$^+$ SuI$^+$ *recD* host (SMR423). Recombinants were assayed on the SuI *recA* host (SMR125) at 42°. Left interval and right interval recombinants were scored by counting turbid and clear plaques, respectively.

RESULTS AND DISCUSSION

Left/ right interval ratios in hotspot crosses: As described (Chapter 2), a measure of how well Chi works in heterology and homology hotspot crosses (see Figure 4-1) is obtained as follows: the titer of the left interval (Chi-stimulated) recombinants is divided by the titer of the right interval (non-Chi-stimulated) recombinants (Chapter 2; Stahl *et al.* 1983). This value is obtained for both Chi$^+$ and Chi$^\circ$ versions of the cross done in parallel. The designs are depicted in Figure 4-1 (A and B). To obtain the hotspot value, the left/right interval value from a Chi$^+$ cross is divided by the left/right interval value from a Chi$^\circ$ cross. This method of calculating

the extent of Chi activity cancels out any Chi-independent factors that can affect the amount of recombination in a Chi-stimulated interval.

Left/right interval ratios are reduced in exonuclease-deficient mutants: In heterology hotspot crosses (depicted in Figure 4-1A), the absence of single-strand-dependent exonucleases, RecJ, ExoI, and ExoVII, reduced the left/right interval ratio in both Chi$^+$ and Chi$^\circ$ crosses (see Table 4-1). In *sbcB15 recJ* and Δ*xonA recJ* cells, the left/right interval ratios in both Chi$^+$ and Chi$^\circ$ crosses were about two-fold lower than the ratios obtained in Exo$^+$ cells. The left/right Chi$^+$ values in *sbcB15 recJ xseA* and Δ*xonA recJ xseA* cells were approximately four-fold lower compared with the values in Exo$^+$; a two-fold depression was observed in the Chi$^\circ$ crosses in the triple-exonuclease-defective strains compared with Exo$^+$. Why are the left/right ratios reduced in the exonuclease-deficient strains?

Heterology crosses with a small interval right of Chi: One hypothesis is that recombination in the left interval is decreased in exonuclease-deficient cells relative to Exo$^+$ cells, because the left interval is smaller than the right interval. Meisel and Roth (1996) suggested that recombination may be low in small intervals when there are no single-strand exonucleases present. This hypothesis predicts that in Exo$^-$ mutants, the left/right ratio would increase and may become equivalent to that of Exo$^+$ when the sizes of the left and right intervals are closer to equal.

To test this hypothesis, I carried out heterology hotspot crosses using phage designs in which the right interval is less than or about equal to the

left interval, and both are very short (around 1kb in length). Phages are shown in Figure 4-1C. Use of the *Ots28* marker in the heterology cross design makes the right interval much shorter (anywhere from 69 to 965 bp-depending on where the *ts* mutation is located in the *O* gene). The left interval is about 1.5 kb.

In the *Ots* heterology hotspot crosses (depicted in Figure 4-1C), I observed a 13-fold increase in the left/right Chi$^+$ ratio and an 18-fold increase in the left/right Chi° ratio in Exo$^+$ cells (Table 4-2) compared with the ratios obtained in Exo$^+$ cells in the *Sam7* heterology crosses (shown in Table 4-1). These results imply that the amount of recombination in the right interval has decreased, and therefore has shifted the ratio up. The decrease in the right interval could be because less length of interval is available in which to complete recombination events.

In the *Ots* heterology hotspot crosses, the left/right Chi$^+$ ratio in *sbcB15 recJ* Δ*xseA* cells was three-fold lower than in Exo$^+$ cells. The left/right Chi° ratio in *sbcB15 recJ* Δ*xseA* cells was two-fold lower than in Exo$^+$. A similar trend was observed comparing Chi$^+$ left/right and Chi° left/right ratios in Δ*xonA recJ* Δ*xseA* with the same ratios in Exo$^+$ (Table 4-2).

The trend with triple-exonuclease-deficient cells *sbcB15 recJ* Δ*xseA* and Δ*xonA recJ* Δ*xseA* is similar to what was observed previously in the *Sam7* heterology crosses[•]. These results are inconsistent with the idea that decreased left/right ratios in Exo$^-$ cells are caused by a decreased ability to recombine in small intervals.

Ots heterology crosses performed in *sbcB15 recJ* and Δ*xonA recJ* double mutants gave surprising results. When comparing the Chi° left/right

ratios of these double Exo$^-$ cells with that in Exo$^+$ cells*, I observed the same reduction as was seen with the *Sam7* heterology crosses (compare Table 4-1 [lines 4, 12] and Table 4-2 [lines 4, 6]). However, the Chi$^+$ left/right ratios in the double mutants were similar to the Chi$^+$ left/right ratios in Exo$^+$. This is unlike what is seen with the triple mutants in the Chi$^+$ crosses. What is the reason for this discrepancy?

One explanation could be that in the Chi$^\circ$ cross, RecBC(D$^-$) could unwind strands in the right interval (because a true Chi does not exist in the Chi$^\circ$ cross, RecBCD may act on a Chi-like sequence in the right interval). Single-strand-dependent exonucleases could then degrade those unwound strands leftward, past the right interval until it reaches the left interval. Recombination could then occur in the left interval. In the absence of all three exonucleases (or even the two main exonucleases RecJ and ExoI) in the Chi$^\circ$ cross, the chances that events initiating in the right interval are resolved in the left interval might be small. Thus, there would be more recombination in the right interval in the absence of single-strand exonucleases than in the left interval. Therefore, the left/right Chi$^\circ$ ratios in double and triple *exo-* cells would still be decreased compared with Exo$^+$ in *Ots* crosses.

My explanation for why left/right Chi$^+$ ratios in *sbcB15 recJ* and Δ*xonA recJ* cells are similar to wildtype Chi$^+$ ratios follows. In the Chi$^+$ cross, most of the left interval recombination events would have had to initiate in the left interval because of RecBCD recognition of a true Chi. Initiation and resolution would all occur in the left interval. In the absence of all three exonucleases, RecJ, ExoI, and ExoVII, recombining in the left interval

* Please see left/right ratios normalized to Exo$^+$ in Tables 4-1 and 4-2.

would not be efficient because of the presence of heterologous DNA directly opposite Chi and preceding the left interval (Chapter 2). However, ExoVII, if present, could allow recombination events to occur past the heterology (support for this is presented in Chapter 2). Therefore, the number of left interval recombinants in a Chi$^+$ cross in *recJ sbcB15* or *recJ* Δ*xonA* cells would not decrease substantially relative to the number in Exo$^+$ cells. Because a decrease in the amount of left interval recombinants is hypothesized not to happen in the double mutants, but decrease of the right interval recombinants is hypothesized to happen (because of *Ots28* - making the right interval short), one would predict an increase in the Chi$^+$ left/right ratio.

FURTHER DISCUSSION

I tested the hypothesis that reduction in left/right Chi$^{+/\circ}$ ratios in exonuclease-deficient mutants is caused by a reduced ability to recombine in small intervals in the absence of exonucleases. This hypothesis is based on an observation by Miesel and Roth (1996). The results presented do not support this interpretation.

Possible effects of heterology in this assay: The reduction of the left/right values in exonuclease-deficient strains compared to Exo$^+$ is not due to the short left interval size per se (Table 4-2). What else could cause the reduced left/right values in exonuclease-deficient strains? In the *Sam7* crosses (Figure 4-1A), there is a heterologous piece of DNA (~3kb) preceding the left interval. Single-strand exonucleases may be required to

degrade this segment of heterologous DNA so that efficient recombination downstream, in the left interval, may take place (see Chapter 2). Therefore, an alternative explanation is that the left interval recombines poorly because of the heterology upstream of it, and that the right interval recombines well because there is no heterology upstream of it. Homology hotspot cross results support this hypothesis. In our homology cross design (see Figure 4-1B) there was no significant decrease in the left/right ratios in both the Chi$^+$ and Chi$^\circ$ crosses when the single-strand exonucleases were lacking (see Table 4-1). These results agree with the heterology hypothesis. Similar findings, but without Chi$^\circ$ control crosses, were reported by Myers *et al.* (1995).

A potential problem with the short right interval cross design is the following: I select against the *Ots28* marker to select for recombination events that occur solely in the right interval (see Figure 4-1C). Although I select for right interval recombinants, some of those recombinants could have had events initiated upstream of the *Ots28* marker and resolved in the right interval. This assay may not be stringent enough to test the effects of interval sizes on recombination when exonucleases are lacking.

In future experiments, crosses should be designed such that a region of heterology precedes the right interval. Recombination machinery may then require the aid of single-strand exonucleases to get across the heterology to recombine in the right interval, in the same way I suggest that it happens in the left interval (Chapter 2). If this is the case, one might obtain a left/right ratio in exonuclease-deficient cells that is very similar to the left/right ratio in Exo$^+$ cells (in both Chi$^+$ and Chi$^\circ$ crosses).

TABLE 4-1.—Crosses diagrammed in Figure 4-1 (A and B) were performed in bacteria of genotypes indicated (see Materials and Methods). A mean ratio of (recombinants in the left interval)/ (recombinants in the right interval) is given for each cross (see Figure 4-1) along with ± values calculated as for standard deviations. Hotspot values are the (ratio of the left interval / right interval in the cross with Chi present) ÷ (ratio of the left interval / right interval in the cross with Chi absent). The hotspot value quantifies the magnitude of Chi activity. Hotspot values of unity indicate no Chi activity. *n*, number of experiments performed. The titer of recombinants in the right interval in Chi$^+$ and Chi$^\circ$ crosses were similar (data not shown). Crosses with the same experiment numbers were performed in parallel. The data presented above are from experiments 1, 7, 15, 16, 17, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, and 37. (See Appendix C-2). The data presented above are part of the data presented in Chapter 2 but are displayed such that (left/right) ratios can be seen. All plaque counts used to generate recombinant titers were greater than 100. het is heterology; hom is homology.

TABLE 4-1 —Ability of Chi to act opposite a heterology in exonuclease-deficient mutants.

Strain	Relevant Genotype	Expt.	*n*	Cross	Average (left/right) ratio		Hot-spot value	left/right ratios normalized to Exo+
122	Exo⁺	1	5	het - χ^+	1.092	± 0.12	14	1.00
				het - χ°	0.07893	± 0.013		1.00
729	*sbcB15 recJ*	1	5	het - χ^+	0.7191	± 0.18	18	0.66
				het - χ°	0.04109	± 0.0096		0.52
122	Exo⁺	2	5	het - χ^+	1.127	± 0.20	15	1.00
				het - χ°	0.0756	± 0.0074		1.00
775	*sbcB15 recJ* Δ*xseA*	2	5	het - χ^+	0.2878	± 0.056	8.4	0.26
				het - χ°	0.0342	± 0.0097		0.45
122	Exo⁺	3	5	het - χ^+	1.038	± 0.19	16	1.00
				het - χ°	0.06693	± 0.0080		1.00
1403	Δ*xonA recJ*	3	5	het - χ^+	0.4813	± 0.12	21	0.46
				het - χ°	0.02289	± 0.0028		0.34
1404	Δ*xonA recJ* Δ*xseA*	3	4	het - χ^+	0.2443	± 0.060	9.5	0.24
				het - χ°	0.02581	± 0.0034		0.39
122	Exo⁺	1	3	hom - χ^+	4.804	± 0.40	15	1.00
				hom - χ°	0.3276	± 0.0041		1.00
729	*sbcB15 recJ*	1	3	hom - χ^+	5.644	± 0.65	16	1.17
				hom - χ°	0.3515	± 0.032		1.07
122	Exo⁺	2	5	hom - χ^+	5.792	± 0.92	18	1.00
				hom - χ°	0.3248	± 0.039		1.00
775	*sbcB15 recJ* Δ*xseA*	2	5	hom - χ^+	5.322	± 0.94	13	0.92
				hom - χ°	0.3974	± 0.028		1.22
122	Exo⁺	3	3	hom - χ^+	5.366	± 0.62	15	1.00
				hom - χ°	0.3648	± 0.020		1.00
1403	Δ*xonA recJ*	3	3	hom - χ^+	7.456	± 1.20	22	1.39
				hom - χ°	0.3352	± 0.012		0.92
1404	Δ*xonA recJ* Δ*xseA*	3	3	hom - χ^+	6.764	± 0.17	18	1.26
				hom - χ°	0.379	± 0.051		1.04

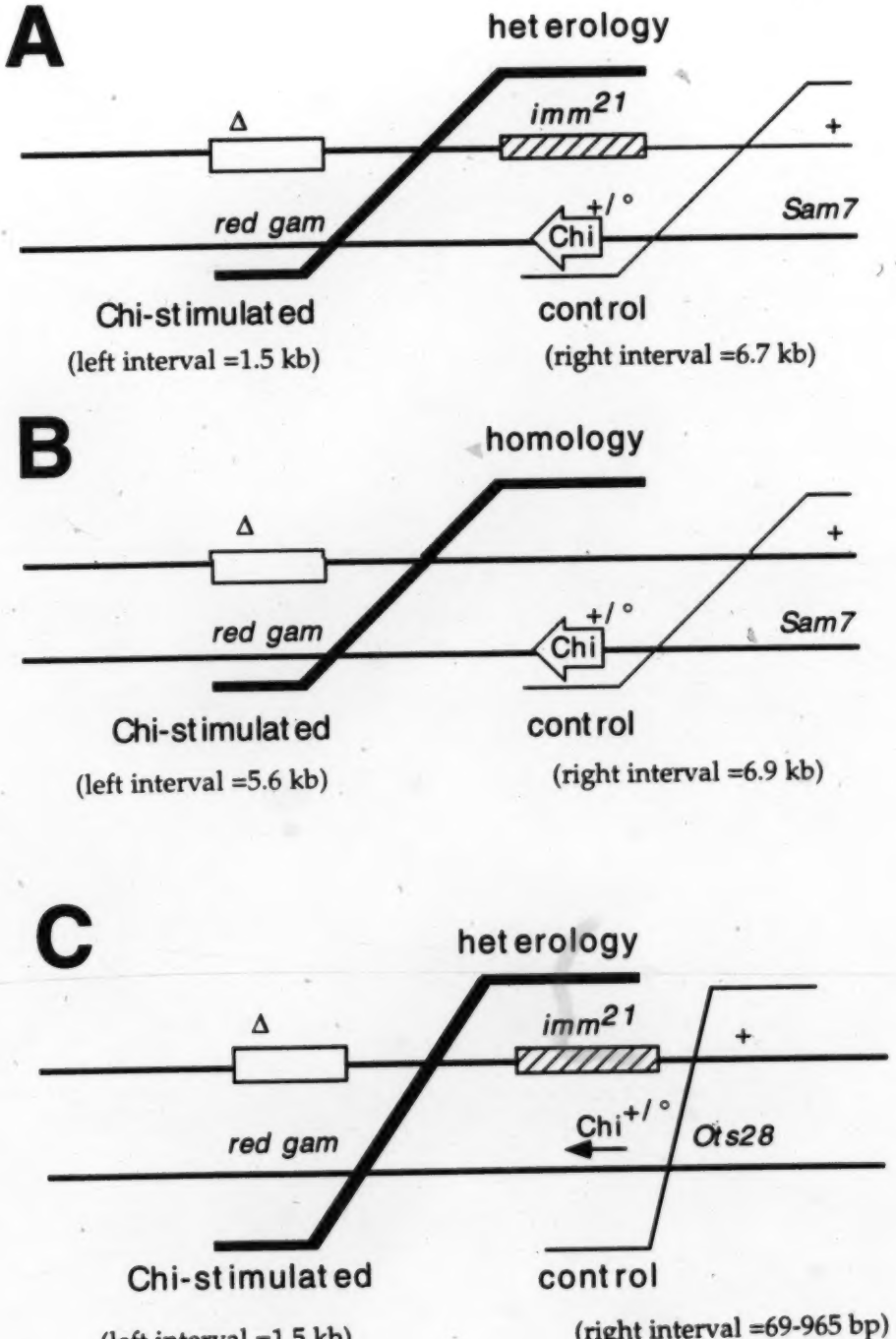
TABLE 4-2

Ability of Chi to act opposite a heterology in single-strand exonuclease-deficient mutants in crosses in which the right interval is small (*Ots* crosses)

Strain	Relevant Genotype	*n*	cross	Average (left/right) ratio	Hot-spot Value	left/right ratios normal-ized to Exo+
122	Exo$^+$	2	het - χ+	13.98 ± 0.70	11	1.00
			het - χ°	1.301 ± 0.16		1.00
729	*sbcB15 recJ*	2	het - χ+	17.04 ± 1.4	21	1.22
			het - χ°	0.7943 ± 0.14		0.61
1403	*ΔxonA recJ*	2	het - χ+	17.23 ± 0.99	45	1.23
			het - χ°	0.3804 ± 0.12		0.29
775	*sbcB15 recJ ΔxseA*	1	het - χ+	4.500	6.8	0.32
			het - χ°	0.6660		0.51
1404	*ΔxonA recJ ΔxseA*	1	het - χ+	5.448	16	0.39
			het - χ°	0.3416		0.26

Heterology crosses with a small right interval were performed (see Figure 4-1C). Data and presentation are as described in Table 4-1. Plaque counts used to generate recombinant titers ranged from 12 to 1169 (See Appendix C-3). Note: the data presented here are preliminary.

FIGURE 4-1.—Hotspot crosses with Chi opposite a heterology. (A) and (B) are as described before (Chapter 2, Figure 2-2). (C) depicts heterology hotspot crosses with a small right interval due to the *Ots28* marker. The genotype of the top λ is λ *Δb1453 imm21* (λHR30). *Δb1453* is a deletion that removes the *red* (λ recombination) genes and the *gam* (inhibitor of RecBCD) gene. *imm21* is a substitution that replaces 4.2kb of λ DNA with approximately 3kb of heterologous DNA. The bottom phage (λHR89 and λHR90) carry *red(amber)270 gam(amber)210* (*red gam* amber mutations), *cIts857*, *Chi*$^{+}$*C* (Chi^{+}-containing parent) or *cII68* (Chi°-containing parent), and *Ots28*. *cIts857* is a temperature sensitive mutation in the *c*I repressor gene. *Ots28* is a temperature sensitive mutation in the *O* (λ replication) gene. The phage in (C) are like the phage in (A) and (B) except for the *Ots28* marker and the lack of the *Eam4* marker on the bottom parent. Each horizontal line represents a duplex λ DNA molecule. The diagonal lines represent regions or intervals in which recombination is selected, with sizes of intervals given underneath. The control (right interval) lengths in cross designs (A) and (B) are 6.7 kb and 6.9 kb respectively. The control (right interval) length in cross design (C) is anywhere from 69-965 bp (depending on where the *ts* mutation is located in the *O* gene).


A
heterology
Δ
imm21
+
red gam
+/°
Chi
Sam7
Chi-stimulated
control
(left interval =1.5 kb)
(right interval =6.7 kb)
B
homology
Δ
+
red gam
+/°
Chi
Sam7
Chi-stimulated
control
(left interval =5.6 kb)
(right interval =6.9 kb)
C
heterology
Δ
imm21
+
red gam
Chi+/°
Ots28
Chi-stimulated
control
(left interval =1.5 kb)
(right interval =69-965 bp)

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5. POTENTIAL ROLE OF EXONUCLEASES IN PUTATIVE RecD LOSS ?

The absence of single-strand exonucleases of opposite polarities, Exonuclease I and RecJ, decreased overall RecBCD-mediated recombination frequency around four-fold relative to Exo$^+$ cells, with the absence of ExoVII contributing only a little (Chapter 2). Why single-strand exonucleases are beneficial in recombination could be that a bit of degradation of one or the other strand of the split-end intermediate, proposed to form by the RecBC(D$^-$) helicase, would allow some of the DNA to remain single-stranded and thus invasive, even if the strands re-anneal.

Although this explanation seems reasonable, alternatives are possible. In the explanation above, the action of single-strand exonucleases takes place after RecD ejection/inactivation. It is plausible that the action of the single-strand exonucleases could occur before RecD ejection perhaps by preparing the DNA substrate that induces the removal of RecD. This chapter explores whether single-strand exonucleases act before or after RecD removal/inactivation.

The idea that single-strand exonucleases may be involved in RecD dissociation is based on the following hypothesis for how RecD becomes dissociated at Chi (S. M. Rosenberg, personal communication). RecD may be susceptible to co-cleavage by an SOS-activated RecA*. Because the substrate that RecA binds to is single-stranded DNA (Kowalczykowski *et. al.* 1994; Clark and Sandler, 1994), the single-strand exonucleases RecJ or ExoI might be required to create this substrate at Chi.

The following observations support the RecA*-mediated cleavage of RecD model: (1) the idea that SOS-induction leads to RecD inactivation is

supported by the observation that SOS-induced cells behaved like RecD$^-$ cells in that Chi activity was partially inhibited (Rinken and Wackernagel, 1992). These observations suggest that an SOS-induced protein assists RecD inactivation and does it so efficiently that almost all ExoV(RecBCD) molecules become RecD$^-$ and Chi is no longer recognized (Rinken and Wackernagel 1992). (2) RecA* has the ability to cleave the LexA and λcI repressors (Kowalczykowski *et al.* 1994) therefore it could have the potential to cleave the RecBCD enzyme. We hypothesize that in normal RecBCD-mediated recombination, a slight SOS induction may give rise to some RecA* proteins which could cleave RecD from RecBC at Chi or a Chi-like sequence. This cleavage could happen preferably at a single-strand region. Single-strand regions at Chi or Chi-like sequences could be generated by single-strand specific exonucleases like RecJ and ExoI.

If single-strand nucleases are needed to create single-stranded regions at Chi in order to facilitate RecA* cleavage of RecD, one might expect the three-to-four-fold drop in recombination frequencies in cells lacking ExoI and RecJ not to be observed in recombination in a *recD* mutant. To test this hypothesis, λ by λ crosses measuring frequencies of RecBCD-mediated recombination were performed in Δ*xonA recJ* or *sbcB15 recJ* cells that also carried a *recD* null allele.

MATERIALS AND METHODS

Bacteria and phage: Bacterial strains used are described in Table 5-1. λ phages for measuring the frequency of RecBCD-mediated recombination were grown from stocks from the laboratory collection. Phage stocks

λHR69, λ*bio1*, and λHR71, λΔ*b2 red3 gamam210 cIts857 Sam7*, were used (Appendix A).

Crosses to measure the frequency of RecBCD-mediated recombination: λ by λ crosses diagrammed in Figure 5-1A were performed as described in Chapter 3 except for the following modifications: lysates collected after the cross were brought up in cesium formate solution to a refractive index of 1.3753. After centrifugation for 24 hours at 30,000 rpm, fractions were collected through the bottom of the tube (Shurvinton *et. al.* 1987; Thaler *et al.* 1989). In Figure 5-1A, Int-generated recombinants contain the longest DNA molecules, therefore they band in the heaviest peak in the gradient. These were assayed on SuIII$^+$ *recA*, SMR137, (selecting against *bio1*). Those Int-generated recombinants that enjoyed a second, gratuitous homologous recombination event were assayed on SuII$^+$ *recA*, SMR125, (selecting against *bio1* and *Sam7*).

RESULTS AND DISCUSSION

The single-strand exonucleases, ExoI and RecJ, are required for full levels of RecBCD-mediated recombination (Chapters 2 and 3). At which intermediate step in recombination do these nucleases act, before or after the formation of RecBC(D$^-$) at Chi? If the action of the nucleases is necessary only for RecD dissociation, then *recD* cells will show no drop in recombination caused by single-strand-dependent exonuclease-deficiency.

λ by λ crosses measuring frequencies of RecBC-mediated recombination were performed in *recD* cells that also contained *recJ sbcB15* or *recJ* Δ*xonA*

mutations, using the method described in Chapter 2, Figure 2-4. The percentage of phage that experienced homologous recombination events out of the total site-specific recombinants was determined (Figure 5-1B).

As reported in Chapter 2, the frequency of homologous recombination in Δ*xonA recJ* cells was about 5-fold lower than Exo$^+$ cells (Figure 5-1B). The frequency of homologous recombination in *sbcB15 recJ* was similar to that in Δ*xonA recJ* (about 3-fold lower). I detected a slight but significant increase in homologous recombination in the *recD* strain as compared with Exo$^+$. The slight increase is indicative of the hyper-recombination activity of *recD* cells. The same magnitude of increase in *recD* cells was also reported by Thaler *et al.* (1989) using similar general recombination/ Int-mediated recombination crosses. Triple mutants, *sbcB15 recJ recD* and Δ*xonA recJ recD*, gave drops in recombination frequency very similar to that seen in their *recD*$^+$ isogenic counterparts (around 2- to 4-fold lower than Exo$^+$). From these results, I conclude that the single-strand exonucleases do not act exclusively in RecD-dissociation. They are required for some other step in recombination that occurs downstream of RecD-loss, and so is independent of *recD*.

The results presented here do not prove or disprove the RecA*-mediated RecD cleavage hypothesis. They merely reveal that the exonuclease effect in recombination is due to some downstream event in the RecBCD recombination pathway, and not due solely to events necessary for RecD cleavage or modification. More direct tests of the RecA* hypothesis are being carried out currently.

TABLE 5-1

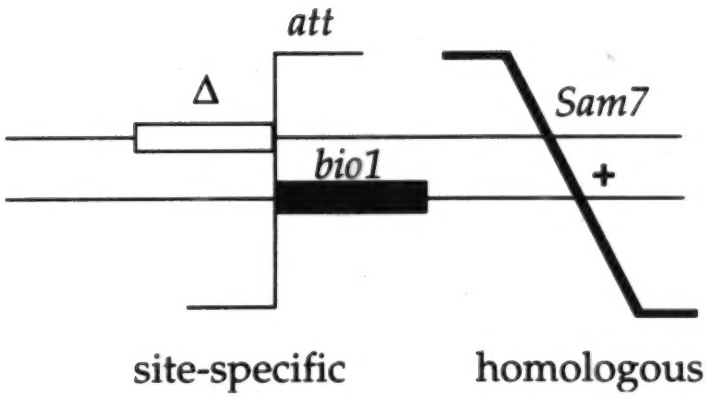
***E.coli* K12 strains**

SMR number	Strain	Relevant genotype	Source or Reference
122	JC11450[1]	Su$^-$ *rec*$^+$	A.J. Clark
125	AFT196	C600[2] (SuII) Δ(*srl-recA*)*306*::Tn*10*	A. Taylor
137	KR3a	SuIII *recA*	*via* F.W. Stahl
423	SMR423[3]	C600 SuIII *recD1903*::*tet*	S.M. Rosenberg
729	HR729	JC11451 (*sbcB15*) *recJ284*::Tn*10*	This work
1403	SZ1403	JC11450 Δ*xonA300*::*Cat* *recJ284*::Tn*10*	Razavy *et al.* (1996)
1554	HR1554	JC11450 *recD6001*::*tet*::*kan*	This work
1558	HR1558	JC11451 (*sbcB15*) *recJ284*::Tn*10* *recD6001*::*tet*::*kan*	This work
1559	HR1559	JC11450 Δ*xonA300*::*Cat* *recJ284*::Tn*10* *recD6001*::*tet*::*kan*	This work

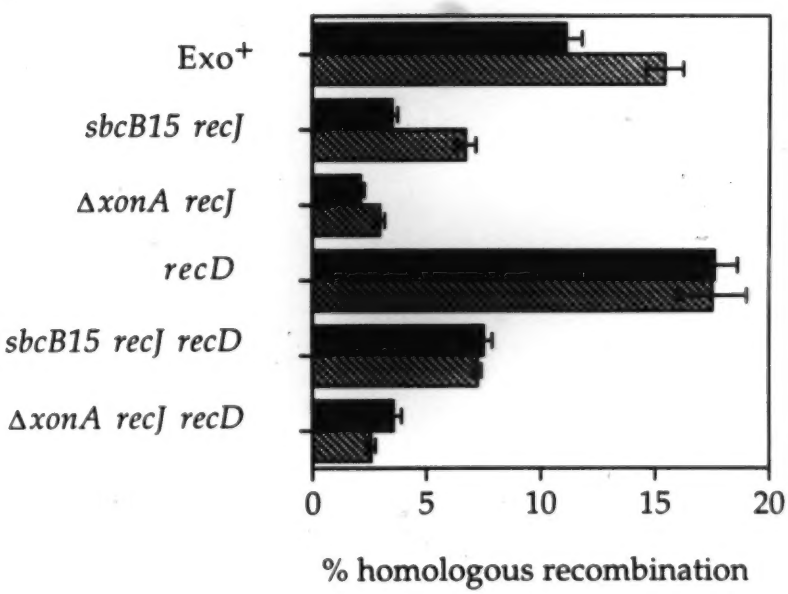
[1]for the background genotype of JC11450 (SMR122) see Table 3-1. SMR729, SMR1403, SMR 1554, SMR1558, and SMR1559 are isogenic derivatives of SMR122.
[2]for background genotype of the C600 derivatives, see Table 3-1.
[3]also carries *hsd rK*$^-$ *mK*$^+$

FIGURE 5-1.—Percent recombination in single-strand exonuclease-defective cells with or without functional *recD*. (A) Crosses measuring frequencies of recombination in the λ right arm. λ genotypes are λ *Δb2 red3 gam210 cIts857 Sam7* (top phage) and λ *Δbio1* (bottom phage). In these crosses, a true Chi site did not exist in the interval in which homologous recombination events were scored. However, recombination events probably take place in our scored interval by the same mechanism as Chi-stimulated recombination using the natural Chi-like sequences of lambda (Cheng and Smith, 1984; Cheng and Smith, 1987). Density gradient fractions containing the site-specific recombinants were plated on SuIII$^+$ *recA* plating host, SMR137, for total phage (*Sam7* and +), and selection for S^+ homologous recombinants from the Int-generated peak of the gradients was done by plating on SuII$^+$ *recA* plating host, SMR125. (B) Each bar represents the mean percent of homologous recombinants in the Int-generated recombinant peak for one cross and density gradient. The black bars represent a set of crosses done in parallel and the gray bars represent a separate set of crosses done in parallel. Error bars represent standard errors of the means derived from the multiple density gradient fractions assayed for total and S^+ phage in each site-specific recombinant peak. See Appendix C6 for data.

A


att
Δ
Sam7
bio1
+
site-specific
homologous

B


Exo+
sbcB15 recJ
ΔxonA recJ
recD
sbcB15 recJ recD
ΔxonA recJ recD
0
5
10
15
20
% homologous recombination

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6. ON THE ROLE OF AN *sbcB* MUTATION IN THE ACTIVATION OF RecF PATHWAY RECOMBINATION IN *E. COLI*.

The main pathway of recombination in *E. coli* is the RecBCD pathway (Kowalczykowski *et al.* 1994). In cells lacking RecBCD, recombination drops 100-to 1000-fold, and the *recBC* cells are sensitive to DNA-damaging agents such as UV light and mitomycin C (Emmerson 1968; Willetts and Mount 1969). DNA-damaging agents induce the SOS response for repair of damaged DNA (Walker 1996). However in *recBC* cells, repair appears to be inefficient probably due both to lack of RecBCD-promoted double-strand break-repair, and to poor SOS induction (Walker, 1996).

A *recBC* cell can regain recombination proficiency and UV-and mitomycin C-resistance by acquiring a null mutation in the *sbcC* gene and a missense mutation called *sbcB15* (Kushner *et al.* 1971; Lloyd and Buckman 1985). This alternative route to recombination and repair is called the "RecF pathway" of recombination because *recF*$^+$ is required for recombination in this pathway as are many other recombination genes (Smith 1987; Sandler and Clark, 1994).

The *sbcB15* mutation, first characterized by Kushner *et al.* (1971), is in the gene encoding Exonuclease I, a 3' to 5' single-strand exonuclease. The activity of Exonuclease I decreases around four-fold in *sbcB15* cells (Kushner *et al.* 1971). However, translational products of the same M_r as wild-type Exo I were observed (Philips *et al.* 1988). It was hypothesized that the reason *sbcB15* suppressed recombination-deficiency in *recBC* cells was that Exonuclease I disrupts some intermediate in the RecF pathway of recombination through its nuclease activity (Horii and Clark 1973; Wang and Smith 1985). In the absence of the nuclease activity, the intermediate

would be able to persist and recombination could take place. Because some residual Exonuclease I activity was observed in *sbcB15* cells (Kushner *et al.* 1971; Kushner *et al.* 1972), the *sbcB15* mutation was thought to be a partial function mutation.

A second mutation, *sbcC* is required in addition to the *sbcB15* allele for suppression of the Rec$^-$ phenotype of *recBC* strains (Lloyd and Buckman 1985). *sbcC*, together with *sbcD*, encodes an ATP-dependent double-strand-dependent endonuclease and exonuclease that attacks DNA hairpins (Connelly and Leach 1997; Gibson *et al.* 1992). *sbcC* mutants can propagate bacteriophage λ carrying long palindromic sequences even in the presence of *recBCD*$^+$ *sbcB*$^+$ (Chalker *et al.* 1988; Gibson *et al.* 1992). *recBC sbcC* mutants are not recombination-proficient in the absence of the *sbcB15* mutation (Lloyd and Buckman, 1985).

Although for many years it was believed that the absence of Exonuclease I (*via sbcB15* mutations) led to the suppression of recombination-deficiency and sensitivity to DNA-damaging agents in *recBC* cells, certain observations were inconsistent with this hypothesis. Kushner *et al.* (1972) characterized another set of mutations in the gene encoding Exonuclease I that suppressed the UV-sensitivity and mitomycin C- sensitivity of *recBC* cells, but not the recombination-deficiency. These mutations were called *xonA*. Because both *sbcB15* and *xonA* mutations produced proteins which possessed reduced exonucleolytic activity (Kushner *et al.* 1972; Philips *et al.* 1988), it was puzzling why *sbcB15*, but not *xonA*, could restore recombination-proficiency in *recBC* cells. Kushner's observation suggested that in *recBC sbcC sbcB* cells, it was not merely the absence of Exonuclease I that suppresses *recBC* recombination

deficiency, but that some other function differing between *sbcB15* and *xonA* mutations might be involved.

Because no known null allele existed, it was difficult to understand the nature of suppression by *sbcB15* and *xonA* alleles, and which, if either class, has a null phenotype. My construction of the Δ*xonA*::*Cat* null allele (Chapter 2) permitted me to examine how *sbcB15* suppresses recombination-deficiency of *recBC sbcC* cells. Discovering a phenotype of *sbcB15* in homology and heterology crosses using the RecBCD pathway (Chapter 2) and finding that the phenotype is dominant (this Chapter) led us to hypothesize a positive function for an *sbcB15*-encoded protein. We propose that SbcB protein binds to 3' DNA ends without degrading them, and that this protects 3' ends from degradation by other nucleases, thereby allowing the ends to be used in recombination. Tests of this hypothesis are presented here.

MATERIALS AND METHODS

Bacteria and phage: Bacterial strains used are described in Table 6-1. λ phages used to measure Chi activity opposite a heterology or homology (Chapter 2) were from the laboratory collection. λ*Ets3 bio1* Δ*nin5* (λHR82) and λΔ*b1453 cIts*857 Δ*nin5 Sam7* (λHR16) were used in crosses to measure frequency of recombination in the RecF pathway (*recBC sbcC sbcB* cells). The *Ets3* phage was obtained from F. Stahl (Sawitzke and Stahl 1992) and the *Sam7* phage was from the laboratory collection.

λ by λ crosses: To measure the ability of Chi to work opposite a homologous or heterologous region of DNA, crosses discussed in Chapter 2 and Chapter 4 were carried out.

To measure the frequency of recombination in *recBC sbcB sbcC* cells and derivatives, standard cross procedure, covered in Chapter 3, was performed with the following modifications. Cross hosts were prepared by inoculating 10µl of each -80°C freezer stock culture into 20ml of MC broth (5mM MgSO4, 0.01mg/ml thymine, 0.01mg/ml vitamin B1 and 50ug/ml guanosine) and incubating (shaking) the flasks at 30°C. The length of time required to grow to ~$1.5x10^8$ depended on the genotype of the culture. *recBC sbcC ΔxonA ΔxseA* and *recBC sbcC ΔxonA ΔxseA ΔxthA* strains have slow growth rates. It took these strains 24 hours to reach a density of ~10^8 cells/ml. In *recBC sbcC lexA71*::Tn*5 sulA* and *recBC sbcC ΔxonA lexA71*::Tn*5 sulA* cells, the optimum density was reached in 12-13 hours. The rest of the strains grew within 8-9 hours. Cells were inoculated and incubated at staggered times so they could be retrieved for the cross at approximately the same time. *recBC sbcC ΔxonA ΔxseA* and *recBC sbcC ΔxonA ΔxseA ΔxthA* cells are extremely filamentous so instead of determining cell concentrations by Petrauf Hauser readings, O.D. readings were taken for all the strains used. Multiplicity of infection (MOI) of the phages used was 0.2 for the *Ets* phage and 10 for the *Sam7* phage (Sawitzke and Stahl 1992). This ensures that every *Ets* phage was given a chance to recombine with the *Sam7* phage. In measuring recombination frequencies, the titer of E^+S^+ recombinants (selected on Su^- *recD* host, SMR264, at 42°C) was divided by the titer of the *Ets* parent (determined by plating on Su^- *recD* host, SMR264, at 30°C).

The titer of the unadsorbed *Ets* phage for each cross was determined by plating on Su⁻ *recD*, SMR264, at 30°C. By comparing this titer with the phage yield at the end of the cross (*Ets* parent, recombinants, *Ets* unadsorbed phage) , an estimate of the extent of the phage burst could be obtained.

RESULTS AND DISCUSSION

Discovering an *sbcB15* phenotype in Chi-stimulated recombination: To test whether single-strand ends are formed at Chi *in vivo*, I performed experiments that assess the ability of Chi to act opposite a heterology or homology in single-strand exonuclease deficient strains (Chapter 2). To do this work, I constructed the first null allele of the gene encoding ExoI, Δ*xonA300::Cat* (referred to as Δ*xonA* for simplicity). *sbcB15*, another allele of the encoding ExoI gene, isolated as a suppressor of *recBC* mutations, was also used. In Δ*xonA* cells, the Chi hotspot value for both heterology and homology crosses was slightly higher than Exo⁺ (Table 2-1; see also Table 6-2). In contrast, *sbcB15* reduced the hotspot activity to about one-half of that of Exo⁺ in both heterology and homology crosses. (Table 2-2; see also Table 6-2). Because the Chi activity in *sbcB15* cells was decreased, and such a decrease was not observed in cells carrying the null allele Δ*xonA*, *sbcB15* may be a dominant, positive function mutation. *sbcB15* might encode a protein that binds to (but does not degrade) 3' ends at Chi, thus blocking the action of other nucleases and/or recombination enzymes on the 3' strand. Through this action, *sbcB15* might reduce Chi activity.

In support of this idea, Phillips *et al.* (1988) showed that *sbcB15* encodes a protein product. This product might retain binding but not nuclease function. It should be noted that even though *sbcB15* depresses Chi activity around 2-fold, it does not affect the overall frequency of RecBCD-mediated recombination (Figure 6-1). In fact, in the frequency assays, *sbcB15* in combination with other single-strand exonucleases gives a pattern of recombination frequencies similar to or more recombination-proficient than those obtained with Δ*xonA* (compare Figure 6-1 with Figure 2-3). Therefore the decrease caused by the single *sbcB15* mutant seems to be specific to recombination stimulated by Chi, or manifests in change to the distribution, not the frequency of recombinants.

***sbcB15* phenotype is dominant in RecBCD-mediated heterology and homology Chi hotspot crosses:** To determine whether *sbcB15* is dominant or recessive in the Chi hotspot assay, an F plasmid containing the wild-type ExoI gene (*sbcB*$^+$) was introduced into Exo$^+$ and *sbcB15* cells. If *sbcB15* is recessive, then introducing a wild-type copy of the ExoI-encoding gene should restore the hotspot value in *sbcB15* cells back to Exo$^+$ levels. This result was not obtained (Table 6-3). The hotspot value obtained in *sbcB15* F'*sbcB*$^+$ cells increased very slightly compared with *sbcB15* cells, but it did not increase to Exo$^+$ levels. Introducing a copy of the *sbcB*$^+$ gene into *sbcB*$^+$ cells had no effect on hotspot values (Table 6-3).

These results indicate that the *sbcB15* allele is either semi-dominant or dominant in the Chi hotspot assay. The two-fold decrease in Chi-activity observed in *sbcB15* cells appears to be due to a positive function provided

by the *sbcB15* allele, the SbcB protein, and not merely due to the absence of Exonuclease I.

***sbcB15* in the RecF pathway:** How does *sbcB15* restore recombination-proficiency in *recBC sbcC* cells? λ by λ crosses (Figure 6-2) were performed in *recBC sbcC* cells containing various alleles of the ExoI gene: *sbcB*$^+$, *sbcB15*, and Δ*xonA*. Recombination frequencies were determined in a λ by λ cross in cells that contain *recBC* mutations using the method of Sawitzke and Stahl (1992).

recBC sbcC sbcB cells are recombination-proficient. *recBC sbcC sbcB*$^+$ cells are expected to have reduced recombination frequencies (Lloyd and Buckman 1985) and this was observed. In Table 6-4, the frequency of recombination in *recBC sbcC sbcB*$^+$ cells decreased about 20-fold compared to *recBC sbcC sbcB*. This confirms previous observations that the *sbcB15* allele is required for suppression of the recombination-deficient phenotype in *recBC sbcC* cells (Lloyd and Buckman 1985). I addressed whether it is the mere absence of ExoI that aids recombination in *recBC sbcC* or whether some positive function of *sbcB15* is involved. If mere absence of ExoI enhances recombination in *recBC sbcC* cells, then the recombination frequencies of *recBC sbcC sbcB* and *recBC sbcC* Δ*xonA* should be similar.

I found that recombination-proficiency of the *recBC sbcC* Δ*xonA* strain decreased by 5-fold compared with *recBC sbcC sbcB* (Table 6-4). Because the *xonA* null allele does not restore recombination-proficiency to a *recBC sbcC* cell, I conclude that the absence of Exonuclease I is not sufficient to

suppress the recombination-deficiency of *recBC* cells. These results also indicate that *sbcB15* is not a null mutation.

Is *sbcB15* dominant or recessive in the RecF pathway?

Because *sbcB15* is dominant in the Chi hotspot assay, a novel function for the *sbcB15* product can be envisioned. Perhaps SbcB protein binds to a 3' end of a split-end intermediate.

Could the same binding activity of SbcB also suppress recombination-deficiency in *recBC sbcC* cells? Our results with *recBC sbcC* Δ*xonA* indicate that *sbcB15* is not a null mutation, therefore *sbcB15* may be dominant to *sbcB*$^+$ in the RecF-mediated recombination assay (Figure 6-2). Previously, Kushner (1971) found that a *recBC sbcC sbcB* strain carrying F' *sbcB*$^+$ had reduced conjugational recombination-proficiency compared with *recBC sbcC sbcB*. However, a control *recBC sbcC sbcB* strain containing a F plasmid without the *sbcB*$^+$ gene, was not used. Therefore, the possibility that the F plasmid alone affects recombination in *recBC sbcC sbcB* cells was not ruled out.

I measured the frequency of λ by λ recombination in *recBC sbcC sbcB* cells that carried either F'*sbcB*$^+$ or F'Δ*xonA* . A 4-fold decrease in recombination in *recBC sbcC sbcB* F'*sbcB*$^+$ was observed compared with *recBC sbcC sbcB* (Table 6-5). The recombination frequency in *recBC sbcC sbcB* F' Δ*xonA* was expected to be similar to the recombination frequency in *recBC sbcC sbcB*, but this was not observed (Table 6-5). Recombination in *recBC sbcC sbcB* F' Δ*xonA* was reduced 9-fold compared with *recBC sbcC sbcB* and reduced 2-fold compared with *recBC sbcC sbcB* F'*sbcB*$^+$. This

latter result implies that the presence of the F', not the presence of the *sbcB*$^+$ gene on it, reduces recombination in this assay.

Alternatively, the F plasmid in our *recBC sbcC sbcB* F'Δ*xonA* strain could have recombined into the chromosome and replaced the chromosomal *sbcB15* allele with the Δ*xonA* allele from the F plasmid *via* a double cross-over event. The event would create a *recBC sbcC* Δ*xonA* strain which is recombination deficient. The second possibility is probably not the case because such a double cross-over event would be (1) rare, and (2) would often either eliminate the *his*::Tn*10* allele on the chromosome, giving rise to a tetracycline sensitivity phenotype, or would eliminate the *his*$^+$ allele on the F plasmid, giving rise to a *his*$^-$ phenotype. Because the cells were both tetracycline resistant and *his*$^+$, a double cross-over event to replace *sbcB15* with Δ*xonA* probably did not happen. On the other hand, conversion of *sbcB15* to Δ*xonA* without associated *his* conversion is possible.

If the F plasmid affects recombination but does so independently of the RecF pathway, the similar recombination frequencies of *recBC sbcC sbcB* F' *sbcB*$^+$ and *recBC sbcC sbcB* F'Δ*xonA* may indicate that *sbcB15* is dominant.

How does *sbcB15* activate recombination in the RecF pathway? 3' single-strand invasions may occur more frequently in the RecF pathway than 5' invasions because RecJ, 5' single-strand exonuclease, is required in this pathway (Lovett and Clark 1983). If the 5' end is degraded by RecJ, it is hard to imagine that end participating in recombination. Conversely, the absence of Exonuclease I activity, even in a *xonA* null strain, can stimulate the RecF pathway (Table 6-4; compare recombination frequency values of

recBC sbcC and *recBC sbcC ΔxonA*). This implies that 3' ends are involved in this pathway. However, results in Table 6-4 show that the absence of Exo I activity is not enough to fully activate the RecF pathway. So how does *sbcB15* activate recombination? One hypothesis is the following: if RecQ, a helicase that is important for this pathway (Nakayama *et al.* 1985), unwinds DNA to produce 3' single-stranded substrates, such substrates may be susceptible to degradation by single-strand exonucleases like Exo I, Exo VII, or even Exo III (Exo III is a double-strand-dependent 3' exonuclease and may degrade 3' ends if strands reanneal; Weiss 1981). Degradation of 3' single-strand ends may prevent them from participating in recombination events. SbcB protein may activate the RecF pathway by binding to 3' single-strand ends and protecting them from degradation by exonucleases like ExoIII and ExoVII. If SbcB protects 3' ends from ExoIII and ExoVII so that the ends may initiate recombination, then by removal of the ExoIII and ExoVII proteins, one might alleviate the requirement for SbcB.

To test this hypothesis, Exonuclease VII and Exonuclease III were removed by mutations (Δ*xseA* and Δ*xthA*, respectively) in *recBC sbcC ΔxonA* cells. If these proteins degrade 3'-end recombination intermediates in the absence of SbcB, then removing them might restore recombination-proficiency.

In *recBC sbcC ΔxonA ΔxseA*, recombination increases to almost the level seen in *recBC sbcC sbcB* cells, almost 5-fold greater than what is seen in *recBC sbcC ΔxonA* (Table 6-4). Addition of the Δ*xthA* gene did not contribute to the restoration of recombination (Table 6-4). These results support the hypothesis that SbcB protects 3' ends from an exonuclease that

can potentially act on those ends. The protection of 3'ends from ExoVII by SbcB could enable ends to have a longer half-life and therefore a greater chance at initiating recombination.

Is recombination increased in the Δ*xseA* derivative because this mutation specifically substitutes for *sbcB15*? Comparing recombination frequencies of *recBC sbcC sbcB* Δ*xseA* cells with *recBC sbcC sbcB* cells should provide the answer. If Δ*xseA* generally elevates recombination, regardless of the presence or absence of *sbcB*, then recombination frequencies in *recBC sbcC sbcB* Δ*xseA* should be higher than *recBC sbcC sbcB*. Unfortunately, I have been unsuccessful in constructing *recBC sbcC sbcB* Δ*xseA* because this genotype appears to be inviable. Therefore further work is required to understand our results with *recBC sbcC* Δ*xonA* Δ*xseA*.

Besides binding ends that recombine, how else could *sbcB15* activate recombination in *recBC* cells? One possibility is that 3' ends stabilized by SbcB act indirectly in recombination by providing an inducing signal for the SOS response. Single-strand DNA activates RecA which cleaves LexA repressor, de-repressing a regulon of ~ 20 recombination and repair genes (Walker 1996). LexA-controlled genes encode proteins required for recombination in *recBC sbcC sbcB* cells such as RuvA, RuvB, RecN, RecQ, and RecA.

However, if SOS functions are necessary for the activation of the RecF pathway, it does not necessarily mean that the induction of SOS is caused by *sbcB15*. To ask whether the induction of the LexA regulon is necessary for the activation of the RecF pathway, *recBC sbcC sbcB lexA3* was constructed. *lexA3* encodes an uncleavable LexA repressor. If the recombination-proficiency caused by *sbcB* relies on induction of the LexA

regulon, then recombination should decrease in *recBC sbcC sbcB lexA3* cells. If Δ*xonA* is unable to fully activate recombination because the inducing signal, single-strand ends, is degraded by other nucleases, then a *recBC sbcC* Δ*xonA lexA*::Tn*5* cell should be recombination-proficient. In *lexA*::Tn*5* cells, the LexA regulon is constituitively de-repressed.

In Table 6-6, the recombination frequency in *recBC sbcC sbcB lexA3* cells dropped by about two-fold compared with its *lexA*$^+$ parent. This indicates that de-repression of a LexA controlled gene(s) is necessary for about half of the recombination restored by *sbcB15*. To test whether *sbcB15* rather than *sbcC* causes the 2-fold requirement for SOS functions, *recBC sbcC lexA3* would be required in our assays. Unfortunately, my attempt to construct *recBC sbcC lexA3* was unsuccessful. Therefore one cannot conclude that SOS functions are required for the *sbcB15* recombination-restoration specifically.

Chua *et al.* (1993) found that *recA* expression increases four-fold in a *recBC sbcC sbcB* strain compared with wild-type. Because *recA* is under SOS regulation, their results imply a role of SOS in the RecF pathway which is consistent with our hypothesis.

In *recBC sbcC lexA*::Tn*5 sulA* cells, recombination is slightly higher than *recBC sbcC* (Table 6-6). This indicates that constituitive LexA de-repression increases recombination-proficiency of *recBC sbcC* cells a bit. However, constituitively derepressing the LexA regulon in *recBC sbcC* Δ*xonA* cells, did not increase recombination; therefore a mechanism other than LexA regulon de-repression must elevate recombination in Δ*xonA* cells (Table 6-6). Interestingly, in *recBC sbcC* Δ*xonA lexA3* cells, the amount of recombination dropped about 2-fold compared with *recBC sbcC*

ΔxonA cells implying that about half the recombination-proficiency conferred by *ΔxonA* requires SOS functions. However, because LexA regulon-induction accounts for only half of the recombination-proficiency in *recBC sbcC sbcB* (or *ΔxonA*) cells (Table 6-6), some other mechanism also contributes.

The results presented in this chapter have illuminated how *sbcB15* works to activate the RecF pathway of recombination. The simple hypothesis that the presence of ExoI disrupts an intermediate of this pathway is not true. A combination of two mechanisms seems to provide *sbcC/sbcB*-mediated activation of recombination. First, LexA regulon de-repression contributes, although its role appears to be partial. Second, the idea that SbcB has a DNA binding function that aids in recombination has been supported by the result that inactivation of ExoVII (3' and 5' single-strand specific exonuclease) increases recombination frequency when *sbcB* is no longer present.

Further experiments need to be done to clarify the emerging picture of how *sbcB* suppression works: (1) crosses with *recBC sbcC lexA3* to determine whether SOS is involved in *sbcB* activation specifically; (2) Crosses with *recBC sbcC sbcB ΔxseA* would ask whether the increase in recombination obtained with *ΔxseA* is specific to "substituting" for *sbcB*. If absence of ExoVII specifically substitutes for *sbcB*, recombination frequencies of *recBC sbcB sbcC ΔxseA* and *recBC sbcC sbcB* should be the same. *recBC sbcC sbcB ΔxseA* should be re-constructed to ascertain this.

The work presented in this chapter sheds light on the mysteries of *sbcB* suppression. An exciting future of discovery lies ahead, in which we can understand the complexities of the recombination mechanisms in *E. coli*.

TABLE 6-1

***E. coli* K-12 strains**

SMR number	Strain	Relevant genotype	Source or Reference
121	JC9387	AB1157[1] Su⁻ *recB21 recC22 sbcB15 sbcC201*	A.J. Clark
122	JC11450[2]	Su⁻ Rec⁺ (AB1157 derivative)	A.J. Clark
123	JC11451	Su⁻ *sbcB15* (AB1157 derivative)	A.J. Clark
124	FS2504	JC11450 *recJ284*::Tn*10*	F. W. Stahl
125	AFT196	C600[3] (SuII) Δ(*srlR-recA*)*306*::Tn*10*	A. F. Taylor
126	AH9	C600 (SuII) *recD* (λ*red3 gam210 imm21cts Sam7*)	A. Hagemann
264	AC417	594[4] (Su⁻) *recD1014*	A. Chaudhury
401	FS1213	SuII *recA* (λ*spi6 Sam7*)	F.W. Stahl
423	SMR423	C600 SuIII *recD1903*::*tet hsdr*$_K^-$*m*$_K^+$	S.M. Rosenberg
729	HR729	JC11451 *recJ284*::Tn*10*	This work
773	SZ773*	AB1157 Su⁻ *sbcB15* Δ(*xseA-gua*) *zff3139*::Tn*10kan*	S. Szigety
774	SZ774*	AB1157 Su⁻ *recJ284*::Tn*10* Δ(*xseA-gua*)*zff3139*::Tn*10kan*	S. Szigety
775	SZ775*	AB1157 Su⁻ *sbcB15 recJ284*::Tn*10* Δ(*xseA-gua*)*zff3139*::Tn*10kan*	S. Szigety
782	HR782	JC11451 *his*::Tn*10* F' *sbcB*⁺ *his*⁺	This work

Table 6-1 (continued)

SMR number	Strain	Relevant genotype	Source or Reference
783	HR783	JC11450 *his*::Tn*10* F'*sbcB*$^+$ *his*$^+$	This work
784	SZ784*	AB1157 Su$^-$ Δ(*xseA-gua*) *zff3139*::Tn*10kan*	Razavy et al. (1996)
839	HR839	JC11450 Δ*xonA300*::*Cat*	Razavy et al. (1996)
1700	HR1700	JC9387 *sbcB*$^+$	This work
1701	HR1701	JC9387 Δ*xonA300*::*Cat*	This work
1775	HR1775	JC9387 Δ*xonA300*::*Cat* Δ(*xseA-gua*) *zff3139*::Tn*10kan*	This work
3108	HR3108	JC9387 Δ*xonA300*::*Cat* Δ(*xseA-gua*) *zff3139*::Tn*10kan* Δ(*xthA-pncA*)::Tn*10*	This work
3150	HR3150	JC9387 *his*::Tn*10* F' *sbcB*$^+$ *his*$^+$	This work
3214	HR3214	JC9387 *his*::Tn*10* F'Δ*xonA300*::*Cat* *his*$^+$	This work
3306	HB17	JC9387 *lexA71*::Tn*5* *sulA211*	Harold Bull
3307	HB18	JC9387 Δ*xonA300*::*Cat* *lexA71*::Tn*5* *sulA211*	Harold Bull
3423	HB20	JC9387*lexA71*::Tn*5* *sulA211*	Harold Bull

Table 6-1 (continued)

SMR number	Strain	Relevant genotype	Source or Reference
3531	HR3531	JC9387 *lexA3*	This work
3553	HR3553	JC9387 Δ*xonA300*::*Cat* *lexA3* *malF*::Tn*10kan*	This work

[1]AB1157 carries the following markers: *thr1 leuB6 thi1 proA his4 argE3 lacY1 galK2 ara14 xyl15 rpsL31 tsx33 Δrac*.
[2]JC11450 is a Su$^-$ derivative of AB1157.
[3]For background genotype of C600, see Table 3-1.
[4]For background genotype of 594, see Table 3-1.
*These strains, spontaneous Su$^-$ derivatives of the strains STL1521, STL1522, STL1605, and STL1607 obtained from S. Lovett (Brandeis Univeristy), were constructed by S.K. Szigety.

TABLE 6-2

Ability of Chi to act opposite a heterology with the *sbcB15* allele of the Exonuclease I-encoding gene

Strain	Relevant Genotype	phage	*n*	Hotspot Value
122	Exo+	heterology	27	14 ± 0.4
123	*sbcB15*	heterology	19	7.3 ± 0.3
839	Δ*xonA*	heterology	7	23 ± 1.4
124	*recJ*	heterology	5	15 ± 1.0
729	*sbcB15 recJ*	heterology	5	18 ± 0.9
122	Exo+	homology	15	14 ± 0.4
123	*sbcB15*	homology	9	7.8 ± 0.4
839	Δ*xonA*	homology	5	16 ± 1.2
124	*recJ*	homology	2	15 ± 2.0
729	*sbcB15 recJ*	homology	3	16 ± 0.5

Crosses of the phage described in Figure 2-2 were performed in each of the genotypes of cells (above). Hotspot values were calculated as the ratio of the (number of recombinants in the Chi-stimulated interval / number of recombinants in the control interval in a Chi$^+$ cross) divided by a similar ratio taken from a Chi$^\circ$ cross done in parallel. Hotspot values (presented as means ± SE) of unity indicate no Chi activity. The extent to which Chi works is seen in the magnitude of the hotspot value greater than one (Stahl *et al.* 1983). These data are from heterology/homology experiments 1-27 that were done for Chapter 2 (Razavy *et al.*, 1996).

TABLE 6-3

Dominance of *sbcB15* in Chi hotspot crosses

Strain	Relevant Genotype	phage	*n*	Hotspot value
122	*sbcB*$^+$	heterology	3	14 ± 0.3
123	*sbcB15*	heterology	3	7.8 ± 0.8
782	*sbcB15* F'*sbcB*$^+$	heterology	3	9.5 ± 0.6
783	*sbcB*$^+$ F'*sbcB*$^+$	heterology	3	14 ± 2.0
122	*sbcB*$^+$	homology	3	14 ± 0.5
123	*sbcB15*	homology	3	7.4 ± 0.5
782	*sbcB15* F'*sbcB*$^+$	homology	3	8.7 ± 0.5
783	*sbcB*$^+$ F'*sbcB*$^+$	homology	3	14 ± 0.3

Data and presentation are as described in Table 6-2. For data, see Appendix C4. Phage stocks used were λHR4, λHR7 and λHR28.

TABLE 6-4

Frequency of Recombination in RecF pathway derivative strains

Strain	Relevant Genotype	*n*	Recombination Frequency Mean ± SE
121	*recBC sbcC sbcB15*	6	1.00 (0.1233 ± 0.0072)
1700	*recBC sbcC*	5	0.053 ± 0.016
1701	*recBC sbcC ΔxonA*	6	0.20 ± 0.024
1775	*recBC sbcC ΔxonA ΔxseA*	3	0.91 ± 0.16
3108	*recBC sbcC ΔxonA ΔxseA ΔxthA*	3	0.93 ± 0.18

Crosses to measure the frequency of recombination using the phage depicted in Figure 6-2 were performed as described in Materials and Methods. The actual recombination frequency mean for *recBC sbcC sbcB* is given in parentheses. Recombination frequencies obtained from *recBC sbcC sbcB* derivative strains are normalized to the recombination frequency obtained for *recBC sbcC sbcB*. *n*, number of experiments. These data are from experiments 1, 3, 4, 5, 6, 7 (see Appendix C5).

TABLE 6-5

Testing the dominance of *sbcB15* in the RecF pathway

Strain	Relevant Genotype	*n*	Relative Recombination Frequency Mean
121	*recBC sbcC sbcB15*	2	1.00 (0.068 ± 0.027)
3150	*recBC sbcC sbcB15* F' *sbcB*$^+$	2	0.24 ± 0.11
3214	*recBC sbcC sbcB15* F' Δ*xonA*	2	0.11 ± 0.0050

Data and presentation are as described in Table 6-4. The ± values are calculated as for standard deviations but with data from 2 independent experiments. The data are from experiments 1 and 2 (see Appendix C5).

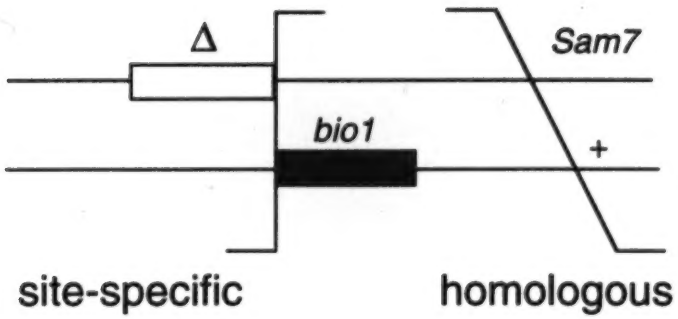
TABLE 6-6

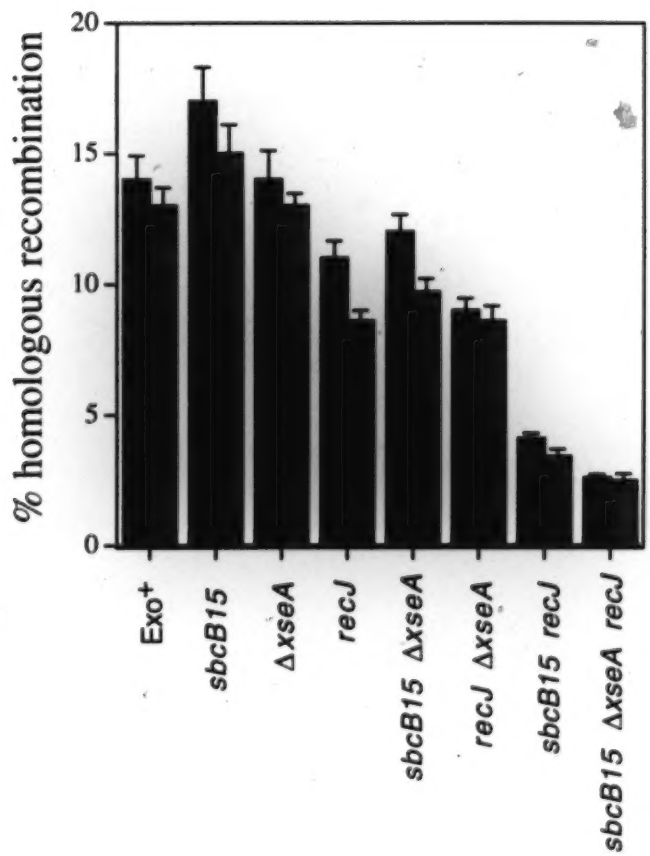
Testing the involvement of the SOS response in *sbcB15*-mediated suppression of recombination-deficiency in *recBC* cells

Strain	Relevant Genotype	*n*	Relative Recombination Frequency Mean
121	*recBC sbcC sbcB15*	2	1.00 (0.1361 ± 0.014)
1700	*recBC sbcC*	2	0.026 ± 0.0075
1701	*recBC sbcC ΔxonA*	2	0.17 ± 0.0050
3531	*recBC sbcC sbcB15 lexA3*	2	0.46 ± 0.030
3553	*recBC sbcC ΔxonA lexA3*	2	0.099 ± 0.010
3306	*recBC sbcC sbcB15 lexA*::Tn*5 sulA*	2	1.05 ± 0.0050
3423	*recBC sbcC lexA*::Tn*5 sulA*	2	0.068 ± 0.018
3307	*recBC sbcC ΔxonA lexA*::Tn*5 sulA*	2	0.13 ± 0.065

Data and presentation are as described in Table 4. The ± values are calculated as for standard deviations but with data from 2 independent experiments. These data are from experiments 6 and 7 (see Appendix C5).

FIGURE 6-1—Percent recombination in single-strand exonuclease-defective cells with *sbcB15*. Crosses measuring recombination in the λ right arm. λ genotypes are λΔ*b2 red3 gam210 cI857 Sam7* (top molecule) and λ Δ*bio1* (bottom molecule). Density gradient fractions containing the site-specific recombinants were plated on SuIII$^+$ *recA*, SMR137, for total phage (*Sam7* and +) and selection for S^+ homologous recombinants from the Int-generated peak of the gradients was done by plating on SuII$^+$ *recA*, SMR125. (*Sam7* is not suppressed by SuII$^+$). Each bar represents the mean percent of homologous recombinants in the site-specific peak for one cross and density gradient. The black bars represent a set of crosses done in parallel and the gray bars represent a separate set of crosses done in parallel. Error bars represent standard errors of the means derived from the multiple density gradient fractions for total and S^+ phage in each site-specific recombinant peak. These data were generated by Sue Szigety. Data from generalized/Int experiments 1 and 2 (May-June 1994).


Δ
Sam7
bio1
+
site-specific
homologous


% homologous recombination
20
15
10
5
0
Exo+
sbcB15
ΔxseA
recJ
sbcB15 ΔxseA
recJ ΔxseA
sbcB15 recJ
sbcB15 ΔxseA recJ

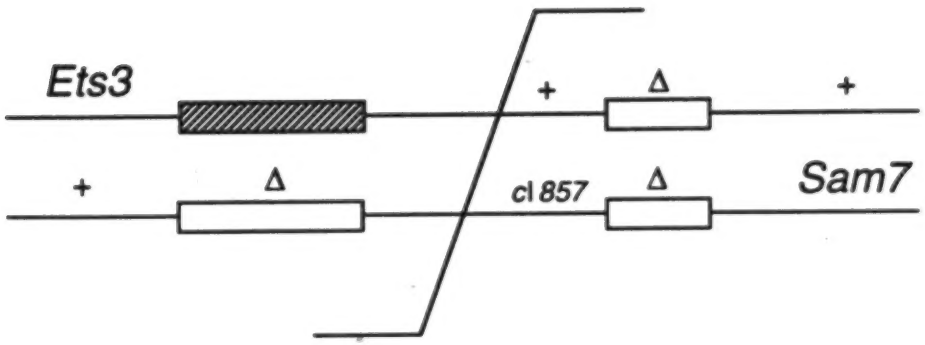


FIGURE 6-2—Crosses to measure the frequency of recombination in *recBC sbcC sbcB* (and derivative) strains. The genotype of the top phage is *Ets3 bio1* Δ*nin5* and the genotype of the bottom phage is Δ*b1453 cI857* Δ*nin5 Sam7*. The phage are unconditionally *red*$^-$ (*via bio1* substitution and Δ*b1453* deletion) so as to inactivate the λ Red recombination system. The phage also contain Δ*nin5* so the analog of the host RecF RecO and RecR proteins (Orf) does not interfere with the host system. RecF, RecO, and RecR are required for the RecF pathway of recombination. Determination of recombination frequencies using these phages are discussed in Materials and Methods. Each horizontal line represents a λ chromosome (duplex DNA molecule). The diagonal line indicates the E^+S^+ recombinant selected.

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7. CONCLUSIONS

This thesis study focused on two specific aims. First, tests of predictions of the split-end model for RecBCD-mediated recombination in *E. coli* were carried out. Single-strand-specific exonucleases were used as tools to elucidate the structure of recombinational intermediates *in vivo*. Second, I examined how the *sbcB15* allele promotes recombination in *recB* cells *via* the RecF recombination pathway.

Are both 5' and 3' ends formed at Chi *in vivo*? The split-end model states that both 5' and 3' ends are formed in a recombinational intermediate, and that either end is capable of invading homologous duplexes to produce recombinants (Rosenberg and Hastings 1991). To determine whether single-strand ends of both polarities are formed at Chi *in vivo*, we used the following strategy: if 5' and 3' single-strand ends exist in recombination intermediates at Chi, then single-strand-specific exonucleases, RecJ, ExoI and ExoVII might affect recombination there. We found that the presence of single-strand-specific exonucleases of either 5' or 3' polarity was necessary for full Chi activity when Chi acted opposite heterologous DNA. The absence of all three nucleases reduced the Chi hotspot value by two-fold (Chapter 2). Because exonucleases of opposite polarities were found to substitute for each other in recombination, these results support the idea that either 5' or 3' single-strand ends can be used in recombination intermediates. The single-strand specific exonucleases may be required to degrade heterologous regions of DNA so that downstream homologous exchanges can take place.

By measuring frequencies of recombination in exonuclease-deficient strains, we found that single-strand-specific exonucleases are also necessary for full levels of recombination in the RecBCD pathway (Chapters 2 and 3). In the absence of heterology, the role of single-strand-specific exonucleases in RecBCD-mediated recombination may be to create and stabilize protruding single-strand ends which can then initiate RecA-mediated invasion. This could be accomplished by degrading a bit of either the 5' end or the 3'end so that if reannealing of the strands occur, a protruding end could still exist and invade (Chapters 2 and 3; Miesel and Roth 1996) Single-strand-specific exonucleases could also be required to stabilize Holliday junctions by degrading the displaced strand of the recipient molecule (see Figure 2-5, and see Corette-Bennett and Lovett 1995). Whether degradation actually takes place on the invading or the invaded molecule *in vivo* remains to be tested.

Chapters 3, 4, 5 extended the analysis on the effects of single-strand exonucleases on Chi-stimulated recombination. (1) I generalized results on recombination frequencies in λ crosses (Chapter 2) to P1 transductional recombination (Chapter 3). I also found that the absence of all three single-strand-specific exonucleases changes the distribution of exchanges that are stimulated by Chi. Surprisingly, this result was also obtained in Chi° crosses. (2) In Chapter 4, I asked whether these nucleases are needed for exchanges in small intervals, and dicussed why this might be so. (3) In Chapter 5, I asked whether the exonucleases play any part in putative RecD-inactivation. The results imply that exonucleases act downstream of the proposed RecD-inactivation stage.

In conclusion, the results with single-strand-exonucleases have illuminated the structure of intermediates in the RecBCD pathway, and have revealed new proteins that were not previously implicated in this pathway.

How does *sbcB15* aid recombination in the RecF pathway? The second aim in this thesis was to understand how *sbcB15* suppression of *recBC* recombination-deficiency works in the RecF recombination pathway. My results with *sbcB15* in the RecBCD pathway suggested that *sbcB15* is a dominant, gain-of-function mutation. To test whether *sbcB15* encodes a positive function in the RecF pathway, I compared *sbcB15* with a null allele $\Delta xonA$::Cat. I found that $\Delta xonA$::*Cat* does not enhance recombination to the same extent as *sbcB15*, although it did increase recombination relative to *sbcB*$^+$. This small increase seemed to be partly dependent on SOS-induction. We envision that *sbcB15* encodes a protein that can bind to, but not degrade 3' single-strand ends. This binding could aid in recombination by protecting 3' ends from the action of other exonucleases. The protected 3' end could then initiate exchange. I presented some evidence in support of this hypothesis.

However, other possible interpretations can be made. For example, *sbcB15* could protect 3' ends which, when left intact in cells, could lead to SOS induction, which can lead to increased expression of recombination/repair genes. Increased expression of SOS recombination/repair genes could alleviate the need for RecBCD for repair. Frequencies of recombination in *recBC sbcB sbcC lexA3* cells were reduced by one-half compared with *recBC sbcB sbcC* cells. *lexA3* encodes

an uncleavable LexA repressor. This indicates that SOS involvement accounts for only one-half of the recombination-enhancing effects of *sbcB15*. Some other mechanism(s) besides SOS induction seems to be responsible for recombination-proficiency of *recBC sbcC* cells carrying an *sbcB15* mutation.

In vitro tests should be performed to test whether the SbcB protein does in fact bind to 3' ends. We chose 3' ends as the substrate for SbcB because 3' ends are the substrates for the wild-type (ExoI) protein. Tests should also be performed to address whether *sbcB15* is dominant in the RecF pathway. Our attempts in this study indicate that the vector used to carry a second copy of the *sbcB* gene, the F', affects recombination. Finally, tests should be carried out to confirm that the *lexA3* effect is specific to *sbcB15* activation of recombination and not to *sbcC*.

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APPENDIX A. COMPLETE COLLECTION OF BACTERIOPHAGE λ STOCKS GROWN OR CONSTRUCTED WHILE IN THE ROSENBERG LABORATORY

HR Collection Number	Genotype	Source	Revertant Frequency
1	*Eam4 redam270 gamam210 cI857 Chi+C Sam7*	λSR4	(S^+) 6.1×10^{-6}
2	*Eam4 redam270 gamam210 cI857 Chi+C Sam7*	λHR4	(S^+) 8.9×10^{-7}
3	*Eam4 redam270 gamam210 cI857 Chi+C Sam7*	λHR2	(S^+) 1.6×10^{-6}
4	*Eam4 redam270 gamam210 cI857 Chi+C Sam7*	λHR1	(S^+) 1.6×10^{-7}
5	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λHR8	
6	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λHR5	(S^+) 2.4×10^{-6}
7	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λHR6	(S^+) 9.7×10^{-7}
8	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λSR5	
9	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λHR7	(S^+) 2.0×10^{-6}
10	*Eam4 redam270 gamam210 cI857 cII68 Sam7*	λHR9	(S^+) 6.1×10^{-7}
11	*Δbio1 Δnin5*	λSR27	$bio^+ < 1.9 \times 10^{-9}$
12	*Δb1453 cI857 Δnin5 Sam7*	λSR37	(S^+) 2.4×10^{-8}

HR#	Genotype	Source	Revertant Frequency
13	*Δb1453 cI857 Δnin5 Sam7*	λHR12	(S^+) 3.3×10^{-6}
14	*Δb1453 cI857 Δnin5 Sam7*	λHR12	
15	*Δb1453 cI857 Δnin5 Sam7*	λHR12	
16	*Δb1453 cI857 Δnin5 Sam7*	λHR15	(S^+) 3.0×10^{-6}
17	*Δb1453 cI857 Δnin5 Sam7*	λHR16	
18	*Δb1453 imm21cts Chi+D*	λSR46	
19	*Δb2 red3 gamam210 cI857 Rts129*	λSR48	(R^+) 4.2×10^{-5}
20	*red3 gamam210 imm21cts Pam80 Δnin5 Chi+D*	λSR66	
21	*Δbio1 imm21cts Pam80 Δnin5 Chi+D*	λHR68	
22	*Δbio1 imm21cts Pam80 Δnin5 Chi+D*	λHR21	(P^+) 7.2×10^{-9}
23	*cos21 Nu1am5 red3 gamam210 cI857 XbaI Sam7*	λHR70	*red+gam+* $<6.4\times10^{-13}$
24	*Δb1453 imm21*	λHR28	$<6.0\times10^{-10}$ on *imm21* lysogen
25	*Δb1453 imm21*	λSR84	
26	*Δb1453 imm21*	λHR25	2.4×10^{-8} on *imm21* lysogen
27	*Δb1453 imm21*	λHR26	$<10^{-8}$ on *imm21* lysogen

HR#	Genotype	Source	Revertant Frequency
28	Δ*b1453 imm21*	λHR27	4.3×10^{-8} on *imm21* lysogen
29	Δ*b1453 imm21*	λHR24	$<6.0 \times 10^{-10}$ on *imm21* lysogen
30	Δ*b1453 imm21*	λHR24	$<3.5 \times 10^{-9}$ on *imm21* lysogen
31	*imm434* clear	λSR101	
32	Δ9 (*att* $^+$ *int*$^-$) cI	λHR33	
33	Δ9 (*att* $^+$ *int*$^-$) cI	λSR136	
34	*Aam32 int4 red3 gamam210* cI26	λSR139	
35	*Aam32 int4 red3 gamam210* cI26 (fully heavy $^{13}C^{15}N$ plate stock)	λHR34	
36	*Aam32 Chi*$^+$*A int4 red3 gamam210* cI26 (fully heavy $^{13}C^{15}N$ plate stock)	λSR140	
37	*Ats14 int4 red3 gamam210* cI26 *Pam80*	λSR142	
38	*int4 red3 gamam210 Chi*$^+$*C Pam80 Rts129*	λHR39	
39	*int4 red3 gamam210 Chi*$^+$*C Pam80 Rts129*	λSR147	

HR#	Genotype	Source	Revertant Frequency
40	*int4 red3 gamam210 cII68 Pam80 Rts129*	λHR41	
41	*int4 red3 gamam210 cII68 Pam80 Rts129*	λSR148	
42	*Oam29 Sam7*	λSR155	
43	*int4 red3 gamam210 Ram5* (with heavy isotopes)	λHR45	
44	*int4 red3 gamam210 Ram5* (with heavy isotopes)	λHR45	
45	*int4 red3 gamam210 Ram5*	λSR157	
46	*cI26*	λSR159	
47	*cI26*	λSR159	
48	*imm21* clear	λSR176	
49	*imm21* clear	λSR176	
50	*gamam210 pal cI857*	SMR189 (induced lysogen)	
51	*Δb1453 cI857 cII68*	λSR52	$1x10^{-11}$ on *recA* strain
52	*Δb1453 cI857 cII68*	λSR233	
53	*int4 red3 gamam210 cI857 Ram5*	λSR238	
54	*int4 red3 gamam210 cI857 Ram5*	λSR238	
55	*Jam5 red3 gamam210 imm434*	λSR241	

HR#	Genotype	Source	Revertant Frequency
56	*Jam5 red3 gamam210 imm434*	λSR241	
57	*Jam5 int4 red3 gamam210 imm434*	λSR244	
58	*Jam5 int4 red3 gamam210 imm434*	λSR244	
59	*red3 gamam210 imm21 Pam80 Chi+D*	λSR261	
60	*Chi+A int4 red3 gamam210 Rts2*	λSR272	
61	*int4 red3 gamam210 Chi+D Rts2*	λSR273	
62	*Ats14 int4 red3 gamam210 cI26 Chi+D*	λSR274	
63	*Ats14 int4 red3 gamam210 cI26 Chi+D*	λSR274	
64	*Ats14 Chi+A int4 red3 gamam210 cI26*	λSR275	
65	*Jam6 Δb1453 cI857 Chi+D*	λSR276	
66	*Δb1453 Chi+D Ram5*	λSR277	
67	*Jam6 Δb1453 Chi+76 cI857*	λSR278	
68	*Δb1453 Chi+76 Ram7*	λSR279	
69	*Δbio1*	λSR293	$bio^+ < 2.3 \times 10^{-9}$
70	*Δb2 red3 gamam210 cI857 Chi+D Rts 129*	λSR294	(R^+) 4.4×10^{-5}

HR#	Genotype	Source	Revertant Frequency
71	*Δb2 red3 gamam210 cI857 Sam7*	λSR295	3.2×10^{-7} on SuII *recA*
72	*Δb527 red3 gamam210 cI857 Chi⁺C Δnin5 Sam7*	λSR324	
73	*Δb527 red3 gamam210 cI857 Δnin5 Sam7*	λSR325	
74	*Δb1453 imm21cts Pam80*	λSR327	
75	*Δb1453 imm21cts Pam80*	λSR327	
76	*palC1 Chi⁺*	λSR325	
77	*palC1 Chi⁺*	λHR76	
78	*Ets3 bio1 Δnin5*	MMS1058 (F. Stahl, U. Oregon)	(E^+) 4.8×10^{-7}
79	*Ets3 bio1 Δnin5*	λHR78 (also λSR434)	
80	*Ets3 bio1 Δnin5*	λHR79	
81	*Ets3 bio1 Δnin5*	λHR78	
82	*Ets3 bio1 Δnin5*	λHR78	$E^+ < 2.8 \times 10^{-10}$
83	*cos2 cosML Δb1453 Chi⁺Z cI857 Oam29 Δnin5*	λSR435	
84	*bio1 Pam80 Chi⁺D (Δnin5?)*	λSR441	(O^+) 7.3×10^{-9}
85	*bio1 Pam80 Chi⁺D (Δnin5?)*	λHR84	

HR#	Genotype	Source	Revertant Frequency
86	*Δb1453 cI857 Oam29 Δnin5*	λHR87	(O^+) 1.3x10^{-8}
87	*Δb1453 cI857 Oam29 Δnin5*	λSR443	
88	*Δb1453 cI857 Oam29 Δnin5*	λHR87	
89	*redam270 gamam210 cI857 Chi$^+$C Ots28*	(λdv (pSR1) x λHR75) x λHR3; pSR1 from SMR1992	(O^+) 4.4x10-5
90	*redam270 gamam210 cI857 cII68 Ots28*	(λdv (pSR1) x λHR75) x λHR10; pSR1 from SMR1992	(O^+) 2.4x10^{-5}
91	*palS gamam210 cI857*	λSKK1 (F. Stahl, U. Oregon)	
92	*palS Δb1453*	λSKK8 (F. Stahl, U. Oregon)	
93	*red3 gamam210 Δnin5 Rts129*	MMS2097 (F. Stahl, U. Oregon)	(R^+) 3.9x10^{-8}
94	*Jts15 red3 gam210 cI857*	MMS2098 (F. Stahl, U. Oregon)	(J^+) 3.6x10^{-7}

APPENDIX B. COMPLETE COLLECTION OF BACTERIAL STRAINS CONSTRUCTED WHILE IN THE ROSENBERG LABORATORY

SMR Collection Number	Strain	Relevant Genotype	Parents & Construction Information
577	HR577	S4[1] *recD6001::mini Tet:: Kan*	#546 cured of λ TSK with heat; Tet^S, Kan^R, UV^R
596	HR596	JC11450[2] *dnaBts sbcB15 recD6001::mini Tet:: Kan*	#534 x P1 (#577); selected Kan^R at 28°C
597	HR597	JC11450 *dnaBts recD6001::mini Tet::Kan*	#535 x P1 (#577); selected Kan^R at 28°C
607	HR607	JC11450 *recBts270 argA*::Tn*10*	#122 x P1 (#581); selected Tet^R; screened for temperature sensitivity.
638	HR638	JC11450 [pBEU2]	$CaCl_2$-transformed #122 with pBEU1 (Prasher *et al.* 1983) prepared from #625 (SK4205); Amp^R; SK4205 from S. Kushner lab, U. Georgia.
639	HR639	JC11450 [pDPK13]	$CaCl_2$-transformed #122 with pDPK13 (Prasher *et al.* 1983) prepared from #626 (SK4258); Amp^R; SK4258 from S. Kushner lab, U. Georgia.

SMR #	Strain	Relevant Genotype	Parents/Source
640	HR640	JC11450 [pDPK15]	$CaCl_2$ transformed #122 with pDPK15 (Prasher *et al.* 1983) prepared from #627 (SK4274); Amp^R; SK4274 from S. Kushner lab, U. Georgia.
642	HR642	JC11450 *xonA2? recB21 argA*::Tn*10*	#644 x P1 (#1); selected Tet^R; UV resistance unrepeatable; presence of *xonA2* questioned.
643	HR643	JC11450 *xonA2? recB1 argA*::Tn*10*	#645 x P1(#1); selected Tet^R; UV resistance unrepeatable; presence of *xonA2* questioned (separate isolate of #642).
644	HR644	JC11450 *xonA2? his*$^+$	#122 x P1(#570); selected His^+; presence of *xonA2* questioned (see #642).
645	HR645	JC11450 *xonA2? his*$^+$	#122 x P1(#570); selected His^+
665	HR665	JC11450 [pDPK20] pBR322 (*xonA*$^+$)	$CaCl_2$ transformed #122 with pDPK20 (Phillips *et al.* 1988) prepared from #628 (SK7743) Amp^R. SK7743 from S. Kushner lab, U. Georgia.
668	HR668	JC11451[3] *sbcB15 mutS201*::Tn*5*	#123 x P1 (#622); selected Kan^R, tested Su^- (spot test); Nalidixic acid resistant.

SMR #	Strain	Relevant Genotype	Parents/Source
669	HR669	JC11451 *sbcB15* [pDPK20] pBR322 (*xonA*$^+$)	$CaCl_2$-transformed #123 with pDPK20 prepared from #628 (SK7743); Amp^R.
670	HR670	JC11451 *sbcB15* [pRDK35] pBR322 derivative.	$CaCl_2$ transformed #123 with pRDK35 (Doherty *et al.*, 1983) prepared from #485; Amp^R
671	HR671	JC11450 [pRDK35]	$CaCl_2$-transformed #122 with pRDK35 prepared from #485; Amp^R.
679	HR679	JC11450 *mutS201*::Tn*5*	#122 x P1 (#622); selected Kan^R
689	HR689	JC11451 *sbcB15* *mutS210*::Tn*5* [pDPK20]	$CaCl_2$ transformed #123 with [pDPK20] from #628; Amp^R.
716	HR716	594 *recD1014* (λ*Oam29* *Sam7*)	Lysogenized #264 with λSR155; screened by spot test with λ *imm*λ (+) and λ *imm21* (-).
728	HR728	JC11451 *sbcB15* *his*::Tn*10*	#123 x P1 (#771); selected Tet^R; *sbcB15* phenotype confirmed by Chi hotspot crosses (Chapter 2). *his*::Tn*10* from Allgood and Silhavy (1991).
729	HR729	JC11451 *sbcB15* *recJ284*::Tn*10*	#123 x P1 (#124); selected Tet^R; to confirm *recJ284*::Tn*10* crossed it into #121 to get UV sensitive strain.

SMR #	Strain	Relevant Genotype	Parents/Source
782	HR782	JC11451 *sbcB15 his*::Tn*10* F'131 (*xonA*$^+$ *his*$^+$)	F'131 from #771 was conjugated into #728; selected His$^+$ Met$^+$.
783	HR783	JC11450 F'131 (*xonA*$^+$ *his*$^+$)	F'131 from #771 was conjugated into #122; selected His$^+$ Met$^+$.
793	HR793	JC11451 *sbcB15 recBD6004*::*mini tet*::*Kan* F'131 (*xonA*$^+$ *his*$^+$)	#782 x P1 (#794); selected Kan^R
794	HR794	S4 *recBD6004*::*mini tet* ::*Kan*	#547 cured of λTSK with heat; Tet^S, Kan^S, screened for UV sensitivity.
838	HR838	JC11450 Δ*xonA300*::*Cat*	#122 x P1 (#842); selected CamR (His$^+$)
839	HR839	JC11450 Δ*xonA300*::*Cat*	#122 x P1 (#842); selected CamR (His$^-$)
842	HR842	C600[4] *recD1009* Δ*xonA*::*Cat*	A 3.8 kb Δ*xonA*::*Cat* fragment from pHR1 (see #3907) used for target replacement of *xonA*$^+$ in #130 (see Chapter 2, Materials and Methods).
1262	HR1262	AB1157 Su$^-$ Δ (*xseA-guaB*) *zff3139*::Tn*10Kan* Δ*xonA300*::*Cat*	#784 x P1(#839); selected Cam^R.
1554	HR1554	JC11450 *recD6001*::*mini tet*::*Kan*	#122 x P1(#577); selected Kan^R; UV resistant.

SMR #	Strain	Relevant Genotype	Parents/Source
1555	HR1555	JC11451 *sbcB15* *recD6001*::*mini tet*::*Kan*	#123 x P1 (#577); selected Kan^R
1556	HR1556	JC11450 Δ*xonA300*::*Cat* *recD6001*::*mini tet*:: *Kan*	#839 x P1 (#577); selected Kan^R
1557	HR1557	JC11450 *recJ284*::Tn*10* *recD6001*::*mini tet*::*Kan*	#124 x P1 (#577); selected Kan^R
1558	HR1558	JC11451 *sbcB15* *recJ284*::Tn*10* *recD6001*::*mini tet*::*Kan*	#729 x P1(#577); selected Kan^R
1559	HR1559	JC11450 *recJ284*::Tn*10* Δ*xonA300*::*Cat* *recD6001*::*mini tet*::*Kan*	#1403 x P1(#577); selected Kan^R
1625	HR1625	JC9937[5] Δ*xonA300*::*Cat*	#173 x P1 (#839); selected Cam^R
1626	HR1626	JC9937 *recD1009* Δ*xonA300*::*Cat*	#131 x P1 (#839); selected Cam^R
1632	HR1632	JC11450 *dnaBts*21	#122 x P1 (AB1157 *dnaBts21 malB*::Tn*9*); Cam^R, temperature sensitive.
1700	HR1700	JC9387[6] *recB21 recC22* *sbcC201*	#121 x P1 (#455); selected His$^+$ *sbcB*$^+$; UV sensitive like #181 (*recB21 recC22*).
1701	HR1701	JC9387 *recB21 recC22* Δ*xonA300*::*Cat sbcC201*	#121 x P1(#839); Cam^R
1775	HR1775	JC9387 *recB21 recC22* Δ*xonA300*::*Cat* Δ (*xseA-guaB*) *zff3139*::Tn*10Kan* *sbcC201*	#1701 x P1(#758); Kan^R Cam^R

SMR #	Strain	Relevant Genotype	Parents/Source
1776	HR1776	JC9387 *recB21 recC22 sbcB15 sbcC201* F'131 (*xonA*$^+$ *his*$^+$)	F'131 (from #771) was conjugated into #121; selected His$^+$ Met$^+$; UV sensitive
1830	HR1830	FA77[7] Su$^-$*dnaBts22* Δ*xonA300*::*Cat*	#87 x P1(#839); Cam^R, temperature sensitive, no growth at 42°C, growth at ≤ 30°C
1933	HR1933	FA77 *dnaBts22* Δ (*xseA-guaB*) *zff3139*::Tn*10Kan*	#87 x P1(#758); Kan^R, *gua*$^-$, temperature sensitive, grows only at ≤30°C.
1992	HR1992	JC11450 [pSR1], pSR1=λdv *imm434 Ots28*	$CaCl_2$-transformed #122 with pSR1 (S. M. Rosenberg) prepared from #46; Kan^R
1993	HR1993	JC11450 [pSR1] Δ*xonA300*::*Cat*	$CaCl_2$-transformed #839 with pSR1 prepared from #46; Kan^R
1994	HR1994	JC11450 *recJ284*::Tn*10* [pSR1]	$CaCl_2$-transformed #124 with pSR1 prepared from #46; Kan^R
1995	HR1995	JC11450 Δ*xonA300*::*Cat recJ284*::Tn*10* [pSR1]	$CaCl_2$-transformed #1403 with #46; Kan^R
2072	HR2072	JC11450 Δ*xonA300*::*Cat recJ284*::Tn*10* Δ (*xseA-guaB*) *zff3139*::Tn*10Kan* [pSR1]	$CaCl_2$-transformed #1404 with #46; selected on plates seeded with λ *imm434* clear (SR101); resistant to λ*imm434* clear but sensitive to λ *imm*λ.

SMR #	Strain	Relevant Genotype	Parents/Source
2210	HR2210	JC9387 *recB21 recC22 sbcB15 sbcC201* F'Δ*xonA300::Cat*	F' Δ*xonA300::Cat* from #3263 was conjugated into #1618; selected His$^+$ Leu$^+$, screened Cam^R.
2445	HR2445	CH5180[8] Δ*xonA300::Cat*	#2439 x P1(#839); Cam^R, *lac*$^-$, Su$^-$.
2446	HR2446	CH5180 *recJ284*::Tn*10*	#2439 x P1(#124); Tet^R, *lac*$^-$, Su$^-$.
2447	HR2447	CH5180 *recJ284*::Tn*10* Δ*xonA300::Cat*	#2443 x P1(#124); Cam^R, Tet^R, lac$^-$, Su$^-$.
2596	HR2596	JC11450 *recB21 argA*::Tn*10* Δ*xonA300::Cat*	#839 x P1 (#580); UV sensitive.
3107	HR3107	JC9387 *recB21 recC22 sbcB15 his*::Tn*10 sbcC201 leu*$^+$	#121 x P1 (#455), then transduced with P1 #728; Tet^R, His$^-$, Leu$^+$
3108	HR3108	JC9387 *recB21 recC22 sbcC201* Δ*xonA300::Cat* Δ(*xseA-guaB*) *zff3139*::Tn*10Kan* Δ(*xthA-pncA*)::Tn*10*	#1775 x P1 (#232); Tet^R, Cam^R, Kan^R.
3150	HR3150	JC9387 *recB21 recC22 sbcB15 his*::Tn*10 sbcC201* F'131(*xonA*$^+$ *his*$^+$)	F'131 from (#783) was conjugated into #3107; selected Leu$^+$ His$^+$ Tet^R.
3204	HR3204	JC9387 *recB21 recC22 sbcB15 sbcC201 recJ284*::Tn*10*	#121 x P1 (#729); Tet^R, UV sensitive; low viability.

SMR #	Strain	Relevant Genotype	Parents/Source
3214	HR3214	JC9387 *recB21 recC22 sbcB15 his*::Tn*10 sbcC201* F'Δ*xonA300*::*Cat his*$^+$	F'Δ*xonA300*::*Cat his*$^+$ from #3263 was conjugated into #3107; selected Leu$^+$ His$^+$ Tet^R.
3263	HR3263	JC11450 F'Δ*xonA300*::*Cat his*$^+$	#783 x P1 (#839); Cam^R marker mated out- this confirms *xonA300*::*Cat* on the F'.
3273	HR3273	JC9387 *recB21 recC22 sbcB15 sbcC201 sulA211 pyrD*$^+$	#121 x P1(#1725) then transduced with P1#1723.
3301	HR3301	JC9387 *recB21 recC22* Δ*xonA300*::*Cat sbcC201 sulA211*	#3273 x P1 (#839); Cam^R
3302	HR3302	C600 *recD1009* (Δ*srl-recA*)306::Tn*10*	#130 x P1 (#624); Tet^R, UV sensitive.
3408	HR3408	JC9387 *recB21 recC22 sbcC201 sulA211*	#3273 x P1 (#455); His$^+$, UV sensitive.
3414	HR3414	JC9387 *recB21 recC22 sbcC201* Δ*xonA300*::*Cat* Δ(*xseA-guaB*) *zff3139*::Tn*10Kan sulA211*	#3301 x P1 (#758); Kan^R, *gua*$^-$, Cam^R; UV sensitive; poor viability.
3438	HR3438	JC9387 *recB21 recC22 sbcC201* Δ*xonA300*::*Cat* Δ(*xseA-guaB*) *zff3139*::Tn*10Kan* Δ(*xthA-pncA*)::Tn*10 sulA211*	#3414 x P1 (#232); Tet^R, *gua*$^-$, Kan^R, Cam^R; slightly UV sensitive; poor viability.
3439	HR3439	Same as #3438 but different isolate	Construction same as #3438.

SMR #	Strain	Relevant Genotype	Parents/Source
3493	HR3493	JC11450 *recJ284*::Tn*10* Δ*xseA18*::*Amp*	#124 x P1 (#3472); Amp^R, Tet^R.
3505	HR3505	JC9387 *recB21 recC22* Δ*xonA300*::*Cat sbcC201* Δ*xseA18*::*Amp sulA211*	#3301 x P1 (#3472); selected Amp^R, also Cam^R, His^-; slightly UV sensitive.
3506	HR3506	JC9387 *recB21 recC22* Δ*xonA300*::*Cat sbcC201* Δ*xseA18*::*Amp* Δ(*xthA-pncA*)::Tn*10 sulA211*	#3505 x P1 (#232); selected Tet^R, also Amp^R, Cam^R; slightly UV sensitive; poor viability.
3507	HR3507	Same as #3506 but different isolate.	Construction same as for #3506.
3508	HR3508	JC9387 *recB21 recC22 sbcB15 sbcC201 sulA211 recA730 srlC300*::Tn*10*	#3273 x P1 (#1955); selected Tet^R; *recA730* confirmed by transducing into #1794 and observing pink lac^+ colonies on MacConkey Lactose plates.
3509	HR3509	JC9387 *recB21 recC22 sbcC201 sulA211 recA730 srlC300*::Tn*10*	#3408 x P1 (#1955); selected Tet^R; *recA730* confirmed by transducing into #1794 and observing pink lac^+ colonies on MacConkey Lactose plates.
3510	HR3510	JC9387 *recB21 recC22* Δ*xonA300*::*Cat sbcC201 sulA211 recA730 srlC300*::Tn*10*	#3301x P1 (#1955); selected Tet^R; *recA730* confirmed by transducing into #1794 and observing pink lac^+ colonies on MacConkey Lactose plates.

SMR #	Strain	Relevant Genotype	Parents/Source
3511	HR3511	JC9387 *recB21 recC22 sbcB15 sbcC201 sulA211 ΔxseA18::Amp*	#3273 x P1 (#3472); selected Amp^R; poor viability.
3512	HR3512	JC9387 *recB21 recC22 sbcB15 sbcC201 sulA211 ΔxseA18::Amp Δ(pncA-xthA)*::Tn*10*	#3511 x P1 (#232); selected Tet^R, also Amp^R; slightly UV sensitive; low viability.
3513	HR3513	Same as #3512 but different isolate.	Construction same as for #3512.
3531	HR3531	JC9387 *recB21 recC22 sbcB15 sbcC201 lexA3*	#3305 x P1 (#817); selected *mal*$^+$, screened Cam^S.
3532	HR3532	Same as #3531 but different isolate.	Constructon same as for #3531.
3533	HR3533	JC9387 *recB21 recC22* (*sbcB*$^+$?) *sbcC201 lexA3*	#3531 x P1 (#455); selected His$^+$; as UV sensitive as #3531 and #3532- *sbcB*$^+$ genotype doubted.
3553	HR3553	JC9387 *recB21 recC22 ΔxonA300::Cat sbcC201 lexA3 malF*::Tn*10Kan*	#1701 x P1 (#2021); selected Kan^R; screened for more UV^S than parents and #3531, #3532.
3593	HR3593	JC9387 *recB21 recC22 sbcC201 lexA3? malF*::Tn*10Kan*	#1701 x P1 (#2021); selected Kan^R; UV^S phenotype unrepeatable-predict to be more UV^S than #3531,#1700, and #817, but this is not observed.

SMR #	Strain	Relevant Genotype	Parents/Source
3594	HR3594	JC9387 *recB21 recC22 sbcB15 sbcC201 ΔxseA18::Amp xseA⁺*	#121 x P1 (#3472); selected Amp^R but this phenotype is unstable. PCR analysis indicates that *xseA* region duplicated and that both *xseA⁺* and *ΔxseA18::Amp* alleles co-exist.
3595	HR3595	JC9387 *recB21 recC22 ΔxonA300::Cat sbcC201 ΔxseA18::Amp*	#1775 x P1 (#3472); selected Amp^R; viability better than #1775; replacement of Δ(*xseA-guaB*) by *xseA18::Amp* observed by PCR analysis.
3596	HR3596	JC9387 *recB21 recC22 ΔxonA300::Cat sbcC201 ΔxseA18::Amp lexA71*::Tn5 *sulA211*	#3505 x P1 (#878); selected Kan^R; *lexA71*::Tn5 confirmed by transducing into #1794 and seeing pink Lac⁺ colonies on MacConkey lactose plates. Kan^R phenotype extremely unstable.
3686	HR3686	JC9387 *recB21 recC22 sbcC201 ΔxseA18::Amp*	#1700 x P1 (#3472); selected Amp^R; more UV sensitive than #1700; low viability.

SMR #	Strain	Relevant Genotype	Parents/Source
3907	HR3907	JC8260 *recB21 recC22 xonA2* [pDPK20; with CAT cassette replacing 630 bp of *xonA*$^+$ gene] = pHR1	630 bp fragment of the *xonA*$^+$ gene in pDPK20 was replaced with CAT cassette from pCAT19 (Fuqua 1992), and plasmid $CaCl_2$ transformed into #630; Cam^R; UV resistant like #630.
3908	HR3908	JC8260 *recB21 recC22 xonA2* [pDPK20] = pBR322 (*xonA*$^+$)	$CaCl_2$-transformed #630 with pDPK20 from #669. Amp^R; UV sensitive like #181.

1 S4 - SuIII
2 JC11450 - Su$^-$ derivative of AB1157 (see Chapters 2, 3, 4, 5)
3 JC11451 - Su$^-$ derivative of AB1157 (also carries *sbcB15*; see Chapters 2,3,4,5)
4 C600 - SuI (see Chapter 3 strain table for reference)
5 JC9937 - Su$^-$ AB1157 background
6 JC9387 - Su$^-$ and also carries *recB21 recC22 sbcB15 sbcC201*
7 FA77 - Su$^-$*dnaBts22*
8 CH5180 - *proC zaj3053*::Tn*10* Δ*lacX74*

Notes:

1- All *recB recC* and/or *recD* alleles were confirmed by plating with λΔ*b1453* Chi$^+$ and Chi$^\circ$ and observing large plaques.

2- Suppressor spot tests were carried out, in almost all strains constructed, to confirm the suppressor status. 10^{-3} dilutions of each λ*Ram5* (λHR45), λ*Sam7* (λHR7) and λAm$^+$ (λHR27) were spotted on bacterial lawns. Su$^-$ strains support the growth of only λAm$^+$, whereas SuII and SuIII strains support the growth of λRam5, and λRam5 and λSam7, respectively.

3-All strains carrying *sbc*C were confirmed by their ability to plate λ*gam*$^-$ carrying a palindrome (λHR92) (Kulkarni and Stahl, 1989).

Plasmid references:

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Prasher, D., D. A. Kasunic, and S. R. Kushner, 1983 Physical and genetic characterization of the cloned *sbcB* (Exonuclease I) region of *Escherichia coli* K-12. J. Bacteriol. **153:** 903-908.

Fuqua, C. W., 1992 Biofeedback. BioTechniques **12:** 224-225.

Other references:

Allgood, N. D. and T. J. Silhavy, 1991 *Escherichia coli xonA* (*sbcB*) mutants enhance illegitimate recombination. Genetics **127:** 671-680.

Kulkarni, S. K., and F. W. Stahl, 1989 Interaction between the *sbcC* gene of *Escherichia coli* and the *gam* gene of phage λ. Genetics **123:** 249-253.

APPENDIX C. FURTHER INFORMATION ON DATA PRESENTED IN CHAPTERS 3, 4, 5, 6.

1. (a) Phage P1 transductions in exonuclease-deficient hosts. Data shown in Figure 3-2.

Experiment #1 (P1 *recQ61*::Tn3; SMR279)

SMR #	Relevant genotype	Ratio of P1 to cells in each transduction	P1[1] plating efficiency with respect to Exo$^+$	# of Amp^R transductants counted.	Transduct. Freq. Amp^R colonies/ P1 phage added	Normal
122	Exo$^+$	1:183	1.0	375	5.68×10^{-5}	1.0
839	Δ*xonA*	1:220	1.2	266	4.03×10^{-5}	0.71
124	*recJ*	1:154	1.2	530	8.03×10^{-5}	1.4
784	Δ*xseA*	1:255	1.4	127	1.92×10^{-5}	0.34
1262	Δ*xonA* Δ*xseA*	1:287	1.6	89	1.35×10^{-5}	0.24
1403	Δ*xonA recJ*	1:142	0.8	200	3.03×10^{-5}	0.53
1404	Δ*xonA recJ* Δ*xseA*	1:236	1.5	80	1.21×10^{-5}	0.21

(6.6×10^6 P1 phage were added per transduction; entire transduction plated)

Experiment #2 (P1 *recQ61*::Tn3; SMR279)

SMR #	Relevant genotype	Ratio of P1 to cells in each transduction	P1 plating efficiency with respect to Exo$^+$	# of Amp^R transductants counted.	Transduct. Freq. Amp^R colonies/ P1 phage added	Normal
122	Exo$^+$	1:37	1.0	1443	5.15×10^{-5}	1.0
839	Δ*xonA*	1:43	0.8	1180	4.21×10^{-5}	0.82
124	*recJ*	1:36	0.9	1588	5.67×10^{-5}	1.1
784	Δ*xseA*	1:53	0.5	1754	6.26×10^{-5}	1.2
774	Δ*xseA recJ*	1:32	0.7	122	4.36×10^{-6}	0.085
1262	Δ*xonA* Δ*xseA*	1:65	1.1	718	2.56×10^{-5}	0.50
1403	Δ*xonA recJ*	1:37	0.9	543	1.94×10^{-5}	0.38
1404	Δ*xonA recJ* Δ*xseA*	1:50	0.8	372	1.33×10^{-5}	0.26

(2.8×10^7 P1 phage were added per transduction; entire transduction plated)

Experiment #3 (P1 *recQ61*::Tn3; SMR279)

SMR #	Relevant genotype	Ratio of P1 to cells in each transduct-ion	P1 plating efficiency with respect to Exo^+	# of Amp^R trans-ductants counted.	Transduct. Freq. Amp^R colonies/ P1 phage added	Normal
122	Exo^+	1:46	1.0	1237	$5.15x10^{-5}$	1.0
839	Δ*xonA*	1:52	0.8	612	$2.55x10^{-5}$	0.50
124	*recJ*	1:37	1.2	1228	$5.12x10^{-5}$	0.99
784	Δ*xseA*	1:87	0.6	1808	$7.53x10^{-5}$	1.5
774	Δ*xseA recJ*	1:15	0.5	445	$1.85x10^{-5}$	0.36
1262	Δ*xonA* Δ*xseA*	1:62	0.4	946	$3.94x10^{-5}$	0.76
1403	Δ*xonA recJ*	1:31	0.6	337	$1.40x10^{-5}$	0.27
1404	Δ*xonA recJ* Δ*xseA*	1:52	0.5	319	$1.66x10^{-5}$	0.26

($2.4x10^7$ P1 phage were added per transduction; entire transduction plated)

Average of Expts 1, 2, 3.

SMR #	*n*	Mean	±SE
122	3	$(5.33x10^{-5})$ 1.0	$(1.4x10^{-6})$
839	3	0.68	0.08
124	3	1.2	0.1
784	3	1.0	0.3
774	2	0.22	0.1
1262	3	0.50	0.1
1403	3	0.39	0.06
1404	3	0.24	0.01

1. (b) Phage P1 transductions in exonuclease-deficient hosts. Data shown in Figure 3-3.

Experiment #4 (P1 *zai2006*::Tn3)

SMR #	Relevant genotype	Ratio of P1 to cells in each transduction	P1 plating efficiency with respect to Exo$^+$	# of Amp^R transductants counted.	Transduct. Freq. Amp^R colonies/ P1 phage added	Normal
122	Exo$^+$	1:37	1.0	329	1.03×10^{-5}	1.0
839	Δ*xonA*	1:48	0.7	271	8.47×10^{-6}	0.82
124	*recJ*	1:40	0.8	160	5.00×10^{-6}	0.49
784	Δ*xseA*	1:60	0.3	424	1.33×10^{-5}	1.3
774	Δ*xseA recJ*	1:23	0.7	146	4.56×10^{-6}	0.44
1262	Δ*xonA* Δ*xseA*	1:55	0.7	180	5.63×10^{-6}	0.55
1403	Δ*xonA recJ*	1:37	0.7	81	2.53×10^{-6}	0.24
1404	Δ*xonA recJ* Δ*xseA*	1:75	0.6	64	2.00×10^{-6}	0.19

(3.2×10^7 P1 phage were added per transduction; entire transduction plated)

Experiment #5 (P1 *zai2006*::Tn3)

SMR #	Relevant genotype	Ratio of P1 to cells in each transduction	P1 plating efficiency with respect to Exo$^+$	# of Amp^R transductants counted.	Transduct. Freq. Amp^R colonies/ P1 phage added	Normal
122	Exo$^+$	1:43	1.0	162	5.40×10^{-6}	1.0
839	Δ*xonA*	1:49	0.6	149	4.97×10^{-6}	0.92
124	*recJ*	1:48	1.1	92	3.07×10^{-6}	0.57
784	Δ*xseA*	1:85	0.9	212	7.07×10^{-6}	1.3
774	Δ*xseA recJ*	1:32	1.2	146	4.87×10^{-6}	0.90
1262	Δ*xonA* Δ*xseA*	1:89	1.2	118	3.93×10^{-6}	0.73
1403	Δ*xonA recJ*	1:51	0.9	74	2.47×10^{-6}	0.46
1404	Δ*xonA recJ* Δ*xseA*	1:77	0.9	32	1.07×10^{-6}	0.20

(3.0×10^7 P1 phage were added per transduction; entire transduction plated)

Average of Expts 4, 5.

SMR #	*n*	Mean	±SD
122	2	(7.85×10^{-6}) 1.0	(2.5×10^{-6})
839	2	0.87	0.05
124	2	0.53	0.04
784	2	1.3	0
774	2	0.67	0.2
1262	2	0.64	0.09
1403	2	0.35	0.1
1404	2	0.20	0.005

[1]In some instances, P1 plating efficiency in a given strain varied between experiments. This is because P1 plaques are difficult to count and a few may have been missed. However, in all exonuclease-deficient strains, a P1 plating efficiency value close to Exo^+ is obtained in two or more experiments. Therefore, they should all be equally receptive to P1.

2. Chi hotspot values. Chi is opposite heterologous or a homologous DNA (*Sam7* crosses). Data from Table 4-1.

The data presented in Table 4-1 are from experiments 7, 15, 16, 17, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, and 37. All experiments except for 37 were a portion of the data presented in Chapter 2. Data for experiment 37 are presented below:

SMR number	Relevant Genotype	Phage	I/II ratios	Hotspot Value
122	Exo$^+$	heterology -χ^+	0.9418	14
		heterology -χ°	0.06677	
123	*sbcB*	heterology -χ^+	0.5925	7.8
		heterology -χ°	0.07589	
729	*sbcB recJ*	heterology -χ^+	0.4929	17
		heterology -χ°	0.02914	

3. Chi hotspot values. Chi is opposite heterologous DNA in crosses in which the non-Chi stimulated interval II is shorter (*Ots28* crosses). Data for Table 4-2.

Experiment #1

SMR #	Relevant Genotype	Phage	# clear plaques	# turbid plaques	Ratio turbids/ clears	Hotspot Value
122	Exo$^+$	heterology -χ^+	88	1169	13.28	9.1
		heterology -χ°	61	89	1.459	
729	*sbcB recJ*	heterology -χ^+	46	718	15.61	17
		heterology -χ°	76	71	0.9342	
1403	Δ*xonA recJ*	heterology -χ^+	48	874	18.21	36
		heterology -χ°	100	50	0.500	
775	*sbcB recJ* Δ*xseA*	heterology -χ^+	34	153	4.500	6.8
		heterology -χ°	36	24	0.6666	
1404	Δ*xonA recJ* Δ*xseA*	heterology -χ^+	49	267	5.449	16
		heterology -χ°	120	41	0.3417	

Experiment #2

SMR #	Relevant Genotype	Phage	# clear plaques	#turbid plaques	Ratio turbids/ clears	Hotspot Value
122	Exo$^+$	heterology -χ^+	12	176	14.67	13
		heterology -χ°	189	216	1.143	
729	*sbcB recJ*	heterology -χ^+	42	776	18.48	28
		heterology -χ°	81	53	0.6543	
1403	Δ*xonA recJ*	heterology -χ^+	29	471	16.24	62
		heterology -χ°	46	12	0.2609	

4. Chi hotspot values. Chi is opposite heterologous DNA in crosses which test dominance of *sbcB*. Data for Table 6-3.

Experiment #1

SMR#	Relevant Genotype	Phage	I/II ratio	HSV
122	*sbcB*$^+$	heterology-χ+	1.266	15
		heterology-χ°	0.08240	
123	*sbcB*	heterology-χ+	0.4321	6.0
		heterology-χ°	0.07196	
782	*sbcB* F' *sbcB*$^+$	heterology-χ+	0.5123	8.6
		heterology-χ°	0.05989	
783	*sbcB*$^+$ F'*sbcB*$^+$	heterology-χ+	1.168	12
		heterology-χ°	0.1015	
122	*sbcB*$^+$	homology -χ+	4.717	13
		homology -χ°	0.3543	
123	*sbcB*	homology -χ+	3.144	6.2
		homology -χ°	0.5087	
782	*sbcB* F'*sbcB*$^+$	homology -χ+	3.469	7.7
		homology -χ°	0.4508	
783	*sbcB*$^+$ F'*sbcB*$^+$	homology -χ+	4.927	15
		homology -χ°	0.3338	

Experiment #2

SMR#	Relevant Genotype	Phage	I/II ratio	HSV
122	*sbcB*$^+$	heterology-χ+	0.8276	14
		heterology-χ°	0.06066	
123	*sbcB*	heterology-χ+	0.5969	7.8
		heterology-χ°	0.07612	
782	*sbcB* F' *sbcB*$^+$	heterology-χ+	0.8117	9.0
		heterology-χ°	0.08980	
783	*sbcB*$^+$ F'*sbcB*$^+$	heterology-χ+	1.211	12
		heterology-χ°	0.100	
122	*sbcB*$^+$	homology -χ+	5.306	14
		homology -χ°	0.3846	
123	*sbcB*	homology -χ+	3.573	7.5
		homology -χ°	0.4789	
782	*sbcB* F'*sbcB*$^+$	homology -χ+	3.730	8.5
		homology -χ°	0.4374	
783	*sbcB*$^+$ F'*sbcB*$^+$	homology -χ+	5.074	14
		homology -χ°	0.3735	

Experiment #3

SMR#	Relevant Genotype	Phage	I/II ratio	HSV
122	*sbcB*$^+$	heterology-χ+	0.9204	14
		heterology-χ°	0.06730	
123	*sbcB*	heterology-χ+	0.6218	9.5
		heterology-χ°	0.06514	
782	*sbcB* F′ *sbcB*$^+$	heterology-χ+	0.7598	11
		heterology-χ°	0.07177	
783	*sbcB*$^+$ F′*sbcB*$^+$	heterology-χ+	1.212	19
		heterology-χ°	0.06280	
122	*sbcB*$^+$	homology -χ+	5.262	15
		homology -χ°	0.3480	
123	*sbcB*	homology -χ+	3.590	8.4
		homology -χ°	0.4270	
782	*sbcB* F′*sbcB*$^+$	homology -χ+	4.036	9.9
		homology -χ°	0.4095	
783	*sbcB*$^+$ F′*sbcB*$^+$	homology -χ+	5.233	14
		homology -χ°	0.3649	

For each separate cross, greater than 150 plaques were counted in each interval (for all experiments). HSV, hotspot value.

5. Crosses in the RecF pathway. Data for Tables 6-4, 6-5, and 6- 6.

Experiment #1

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Frequency (absolute)	Recomb Frequency (normalized)
121	*recBC sbcC sbcB*	9.35×10^6	8.85×10^5	0.09465	1.00
1700	*recBC sbcC*	1.96×10^6	9.50×10^3	0.004847	0.051
1701	*recBC sbcC ΔxonA*	5.02×10^6	1.55×10^5	0.03088	0.33
1775	*recBC sbcC ΔxonA ΔxseA*	1.52×10^6	1.82×10^5	0.1197	1.3
3108	*recBC sbcC ΔxonA ΔxseA ΔxthA*	1.47×10^6	2.00×10^5	0.1361	1.4
3150	*recBC sbcC sbcB* F'*sbcB*$^+$	6.51×10^6	2.16×10^5	0.03318	0.35
3214	*recBC sbcC sbcB* F' *ΔxonA*	2.76×10^6	2.74×10^4	0.009928	0.11

>76 plaques counted for generating each titer.

Experiment #2

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	1.78×10^6	7.28×10^4	0.04090	1.00
3150	*recBC sbcC sbcB* F'*sbcB*$^+$	2.10×10^6	1.15×10^4	0.005476	0.13
3214	*recBC sbcC sbcB* F' *ΔxonA*	8.90×10^5	3.65×10^3	0.004101	0.10

>318 plaques counted for generating each titer.

Experiment #3

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	2.01×10^7	2.77×10^6	0.1378	1.00
1701	*recBC sbcC ΔxonA*	7.83×10^6	1.86×10^5	0.02375	0.17
1775	*recBC sbcC ΔxonA ΔxseA*	8.58×10^5	8.88×10^4	0.1035	0.75

>343 plaques counted for generating each titer.

Experiment #4

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	1.60×10^7	1.83×10^6	0.1144	1.00
1700	*recBC sbcC*	1.02×10^6	5.00×10^3	0.004902	0.043
1701	*recBC sbcC ΔxonA*	1.15×10^7	2.22×10^5	0.01930	0.17
3108	*recBC sbcC ΔxonA ΔxseA ΔxthA*	1.83×10^6	1.56×10^5	0.08525	0.75

>200 plaques counted for generating each titer.

Experiment #5

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	2.42×10^7	2.92×10^6	0.1207	1.00
1700	*recBC sbcC*	4.16×10^6	6.03×10^4	0.01450	0.12
1701	*recBC sbcC ΔxonA*	7.30×10^6	1.78×10^5	0.02438	0.20
1775	*recBC sbcC ΔxonA ΔxseA*	1.19×10^6	1.00×10^5	0.08403	0.69
3108	*recBC sbcC ΔxonA ΔxseA ΔxthA*	2.09×10^6	1.67×10^5	0.07990	0.66

>241 plaques counted for generating each titer.

Experiment #6

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	1.67×10^7	2.51×10^6	0.1503	1.00
1700	*recBC sbcC*	9.15×10^5	2.48×10^3	0.002710	0.018
1701	*recBC sbcC ΔxonA*	7.90×10^6	1.91×10^5	0.02418	0.16
3531	*recBCsbcC sbcB lexA3*	1.64×10^7	1.07×10^6	0.06524	0.43
3553	*recBC sbcC ΔxonA lexA3*	9.33×10^6	1.25×10^5	0.01340	0.089
3306	*recBC sbcC sbcB lexA*::Tn*5 sulA*	1.30×10^7	2.04×10^6	0.1569	1.04
3423	*recBC sbcC lexA*::Tn*5 sulA*	2.39×10^6	1.81×10^4	0.007573	0.050
3307	*recBC sbcC ΔxonA lexA*::Tn*5 sulA*	2.80×10^6	2.53×10^4	0.009036	0.060

>99 plaques counted for generating each titer.

Experiment #7

SMR#	Relevant Genotype	Titer S^+ and recombinants	Titer recombinants	Recomb. Freq. (absolute)	Recomb Freq. (normalized)
121	*recBC sbcC sbcB*	1.56×10^7	1.90×10^6	0.1218	1.00
1700	*recBC sbcC*	6.70×10^5	2.68×10^3	0.004000	0.033
1701	*recBC sbcC ΔxonA*	6.85×10^6	1.41×10^5	0.02058	0.17
3531	*recBCsbcC sbcB lexA3*	7.75×10^6	4.68×10^5	0.06039	0.49
3553	*recBC sbcC ΔxonA lexA3*	4.59×10^6	6.10×10^4	0.01330	0.109
3306	*recBC sbcC sbcB lexA*::Tn*5 sulA*	5.08×10^6	6.48×10^5	0.1275	1.05
3423	*recBC sbcC lexA*::Tn*5 sulA*	1.59×10^6	1.66×10^4	0.01044	0.086
3307	*recBC sbcC ΔxonA lexA*::Tn*5 sulA*	1.28×10^6	2.99×10^4	0.02336	0.19

>107 plaques counted for generating each titer.

6. Crosses to determine frequencies of recombination in exonuclease-deficient mutants. Frequencies of generalized RecBCD-mediated recombination/ Int-mediated recombination. Data used to generate Figure 5-1.

Experiment #1 (also see expanded data and graphs in the pages following)

Strain	Relevant Genotype	*n*	First plating		Second plating		Average	
			% gen /Int	±SE	% gen /Int	±SE	% gen /Int	±SE
SMR122	Exo$^+$	9	14	0.9	17	0.6	15	0.8
SMR729	*sbcB recJ*	9	6.1	0.5	7.4	0.4	6.7	0.5
SMR 1403	Δ*xonA recJ*	9	2.9	0.2	_	_	2.9	0.2
SMR1554	*recD*	6	17	1.2	18	1.8	18	1.5
SMR1558	*sbcB recJ recD*	4	7.3	0.2	_	_	7.2	0.2
SMR 1559	Δ*xonA recJ recD*	5	2.5	0.2	_	_	2.6	0.2

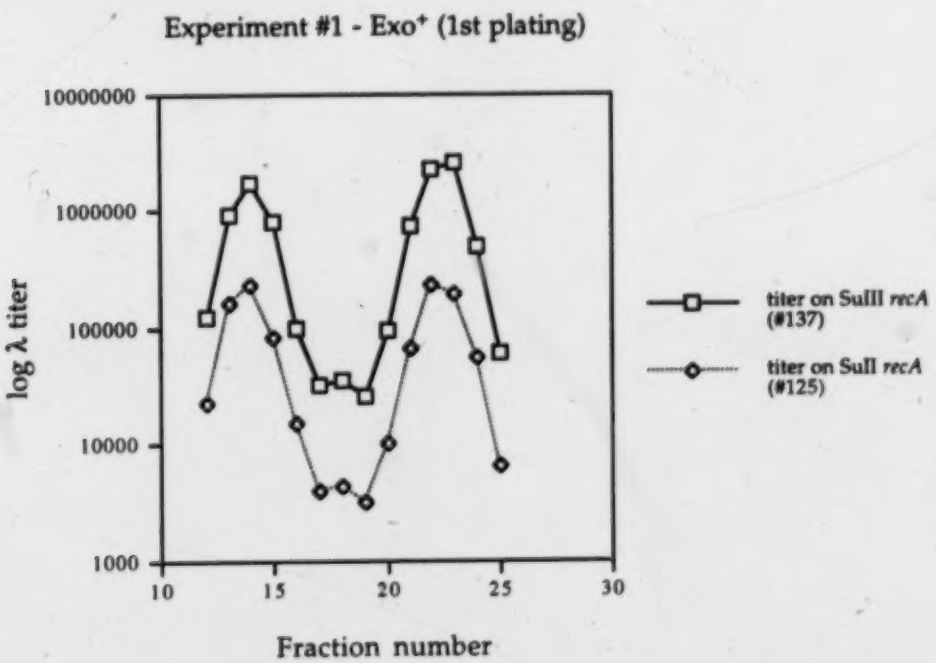
Note: >70 plaques were counted for each fraction in this experiment.

Experiment #2 (also see expanded data in the pages following)

Strain	Relevant Genotype	Average	
		% gen /Int	±SE
SMR122	Exo$^+$	11	0.8
SMR729	*sbcB recJ*	3.2	0.2
SMR 1403	Δ*xonA recJ*	2.1	0.2
SMR1554	*recD*	18	0.9
SMR1558	*sbcB recJ recD*	7.5	0.4
SMR 1559	Δ*xonA recJ recD*	3.5	0.4

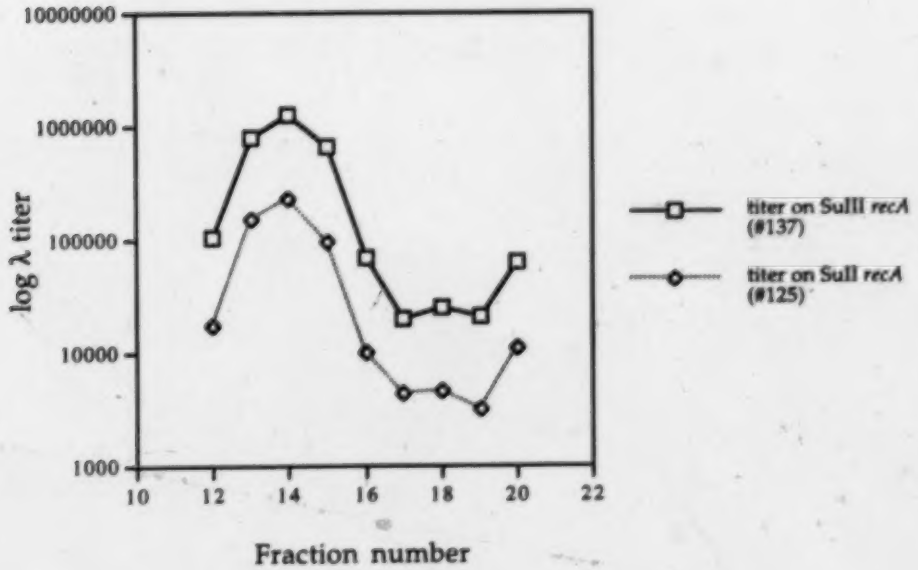
Note: >80 plaques were counted for each fraction in this experiment.

Data used to generate Figure 5-1 (Generalized RecBCD-mediated recombination/ Int-mediated recombination density gradients).



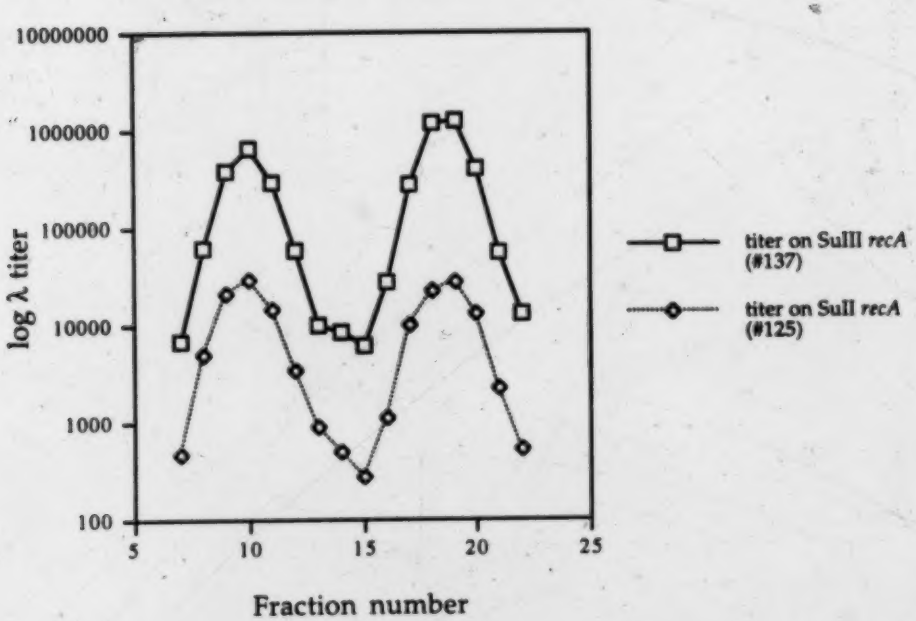
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
12	125000	22400	18	14	0.9
13	927000	164000	18		
14	1710000	235000	14		
15	807000	84000	10		
16	99000	15300	15		
17	32900	4000	12		
18	36300	4470	12		
19	26100	3170	12		
20	94000	10300	11		
21	760000	65700			
22	2290000	236000			
23	2590000	197000			
24	508000	54800			
25	61400	6500			

Experiment #1 - Exo$^+$ (2nd plating)



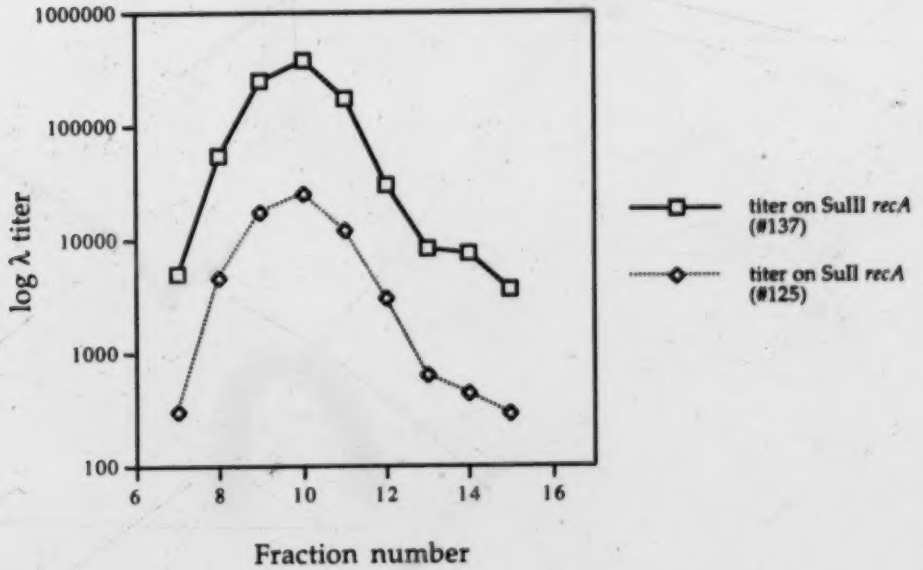
Fraction #	Titer on #137 (int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	± SE
12	105000	17400	17	17	0.6
13	807000	153000	19		
14	1290000	235000	18		
15	660000	97700	15		
16	68700	10000	15		
17	20400	4380	21		
18	25300	4580	18		
19	21400	3130	15		
20	64300	10900	17		

Experiment #1 - *sbcB recJ* (1st plating)



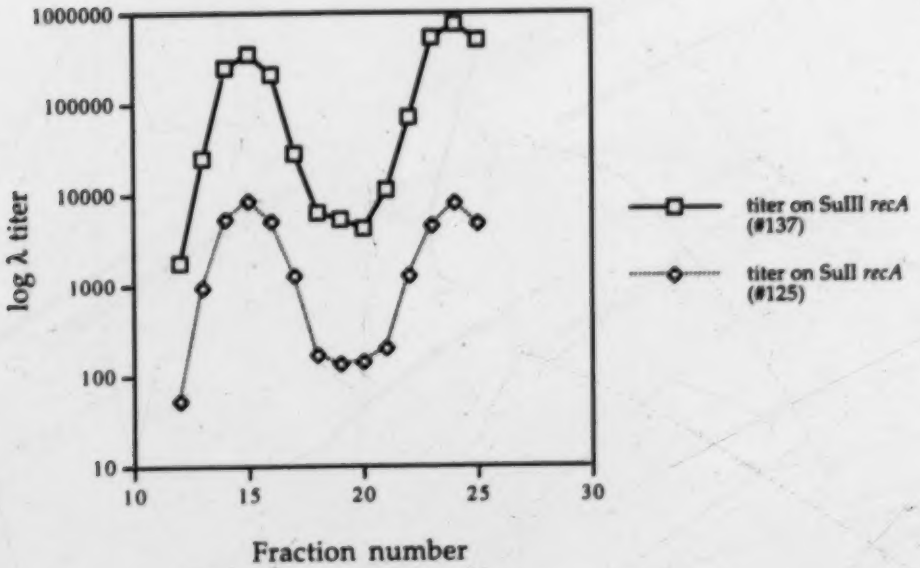
Fraction#	Titer on #137 (int)	Titer on #125 (gen)	(gen/int) x100	Average gen/int	±SE
7	7000	485	6.9	6.1	0.5
8	62000	5170	8.3		
9	398000	21800	5.5		
10	663000	29500	4.5		
11	303000	14500	4.8		
12	61000	3430	5.6		
13	10400	930	8.9		
14	8800	495	5.6		
15	6350	280	4.4		
16	28600	1110			
17	283000	10200			
18	1230000	23100			
19	1250000	27600			
20	403000	13700			
21	57500	2300			
22	13200	545			

Experiment #1 - *sbcB recJ* (2nd plating)



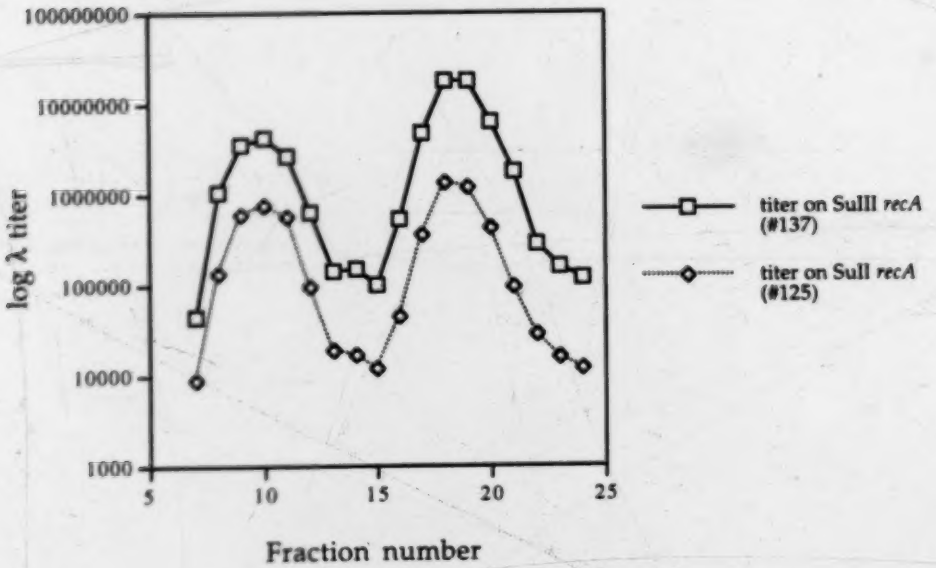
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/int	±SE
7	4970	300	6	7.4	0.4
8	54300	4600	8.5		
9	250000	17600	7		
10	383000	24900	6.5		
11	173000	12000	6.9		
12	30500	3040	10		
13	8200	635	7.7		
14	7500	445	5.9		
15	3700	285	7.7		

Experiment #1 - Δ*xonA recJ*



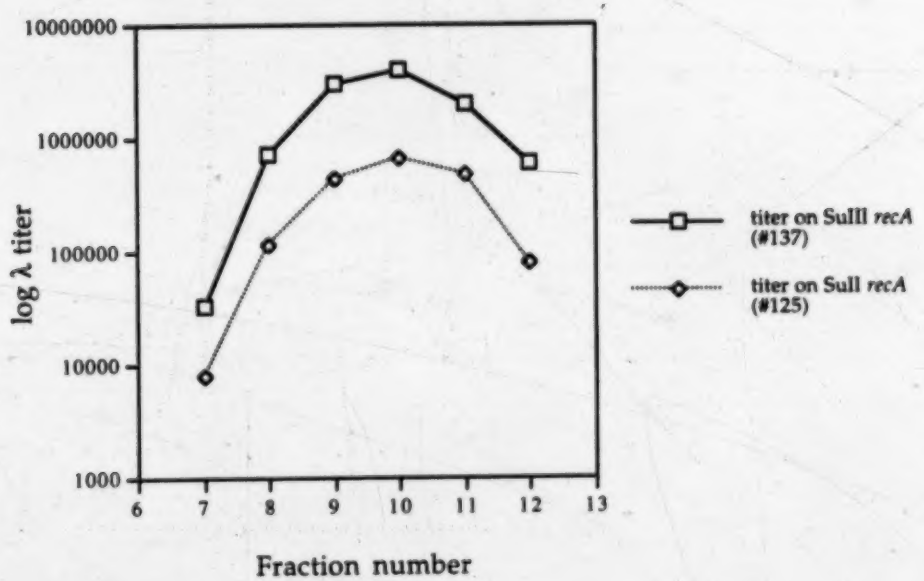
Fraction#	Titer on #137 (int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	±SE
12	1730	54	3.1	2.9	0.2
13	25300	935	3.7		
14	246000	5370	2.2		
15	344000	8250	2.4		
16	215000	4870	2.3		
17	28000	1230	4.4		
18	6150	164	2.7		
19	5350	135	2.5		
20	4300	138	3.2		
21	10900	196			
22	69000	1240			
23	533000	4420			
24	747000	7960			
25	493000	4700			

Experiment #1 - *recD* (1st plating)



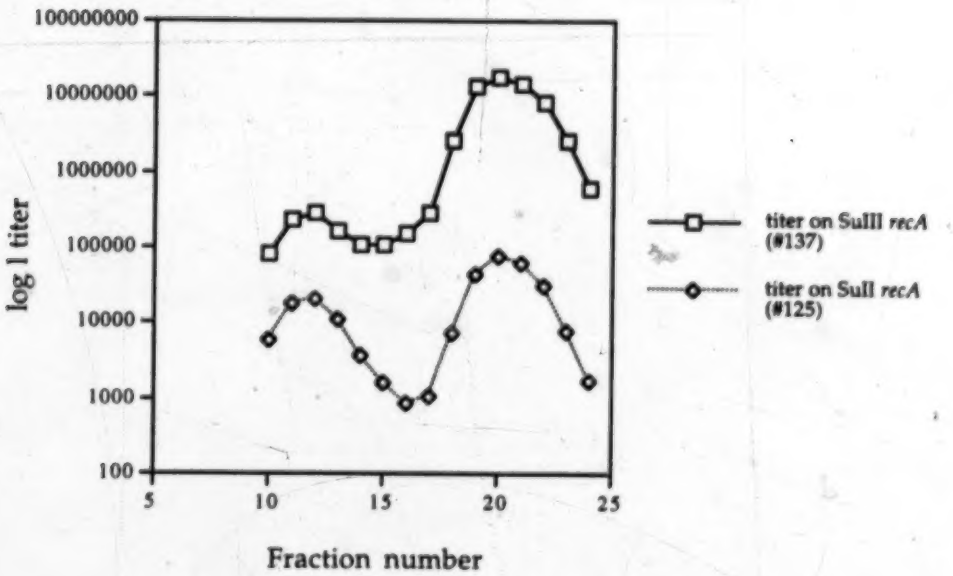
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
7	44000	8900	20	17	1.2
8	1050000	134000	13		
9	3500000	580000	17		
10	4200000	730000	17		
11	2630000	568000	22		
12	630000	93000	15		
13	139000	18400			
14	152000	16900			
15	100000	12100			
16	530000	44400			
17	4800000	344000			
18	17800000	1360000			
19	17800000	1210000			
20	6350000	413000			
21	1810000	93000			
22	273000	27600			
23	157000	18100			
24	120000	11700			

Experiment #1 - *recD* (2nd plating)



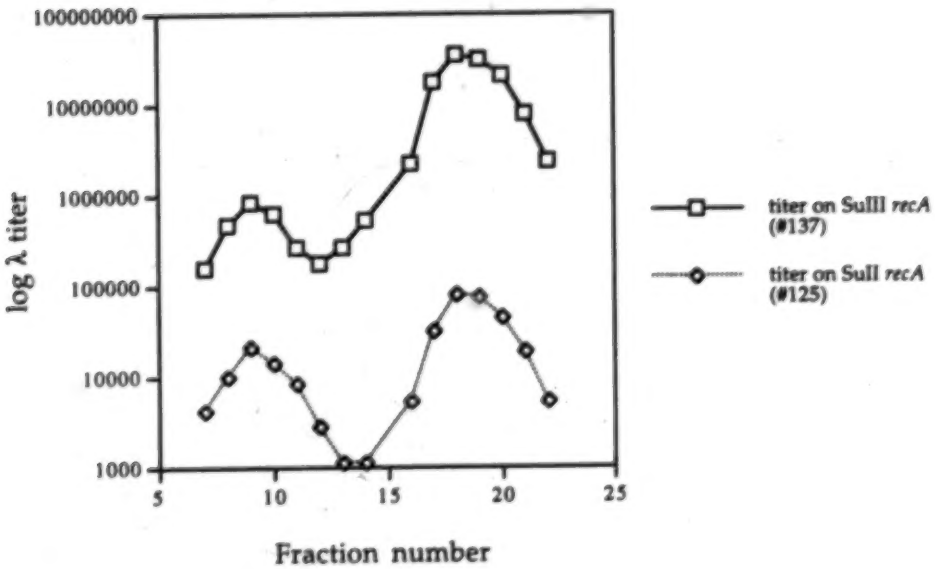
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	±SE
7	33200	7900	24	18	1.8
8	725000	117000	16		
9	3060000	428000	14		
10	4020000	670000	17		
11	2040000	483000	24		
12	610000	78500	13		

Experiment #1 - *sbcB recJ recD*



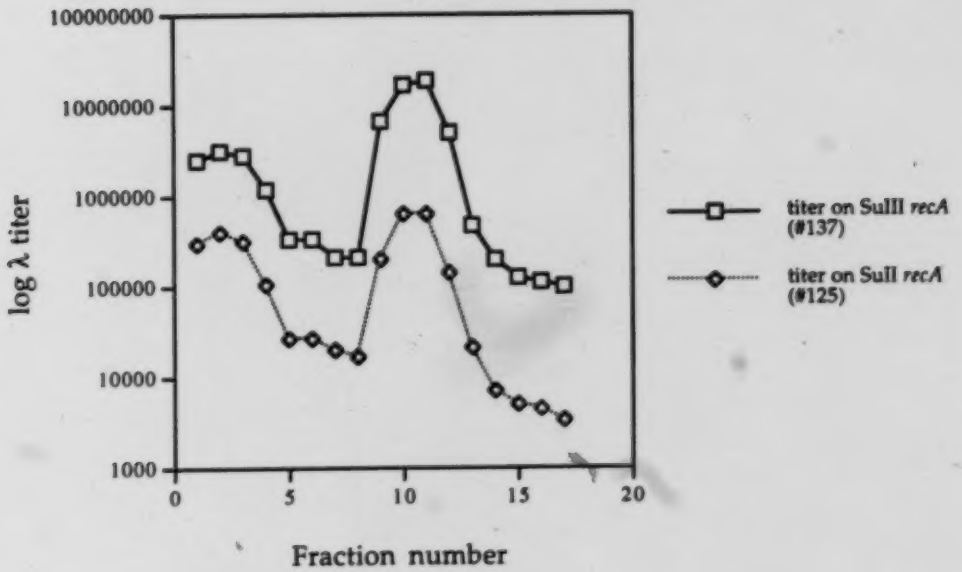
Fraction #	Titer on #137 (int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	±SE
10	80700	5850	7.3	7.3	0.2
11	231000	17800	7.7		
12	282000	20500	7.3		
13	168000	11300	6.7		
14	104000	3670			
15	109000	1630			
16	152000	827			
17	290000	1030			
18	2610000	7050			
19	13900000	44500			
20	18400000	75000			
21	14200000	63000			
22	8500000	30700			
23	2540000	8050			
24	585000	1670			

Experiment #1 - *recJ ΔxonA recD*



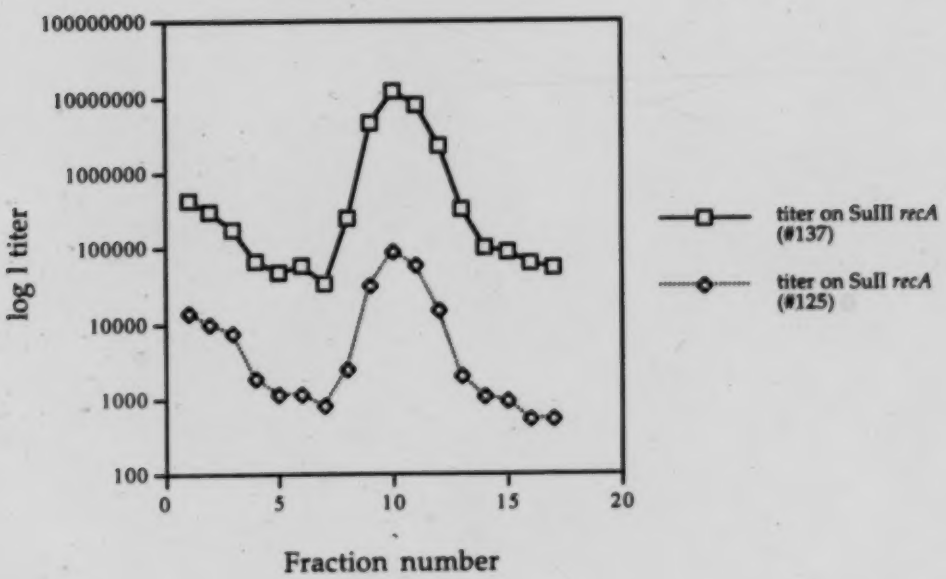
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
7	153000	4280	2.8	2.5	0.2
8	467000	10000	2.1		
9	845000	20600	2.4		
10	632000	14100	2.2		
11	257000	8300	3.2		
12	176000	2860			
13	270000	1130			
14	520000	1090			
15					
16	2180000	5240			
17	17800000	30900			
18	35300000	78300			
19	30700000	74000			
20	20300000	44300			
21	7700000	19000			
22	2300000	5130			

Experiment #2 - Exo$^+$



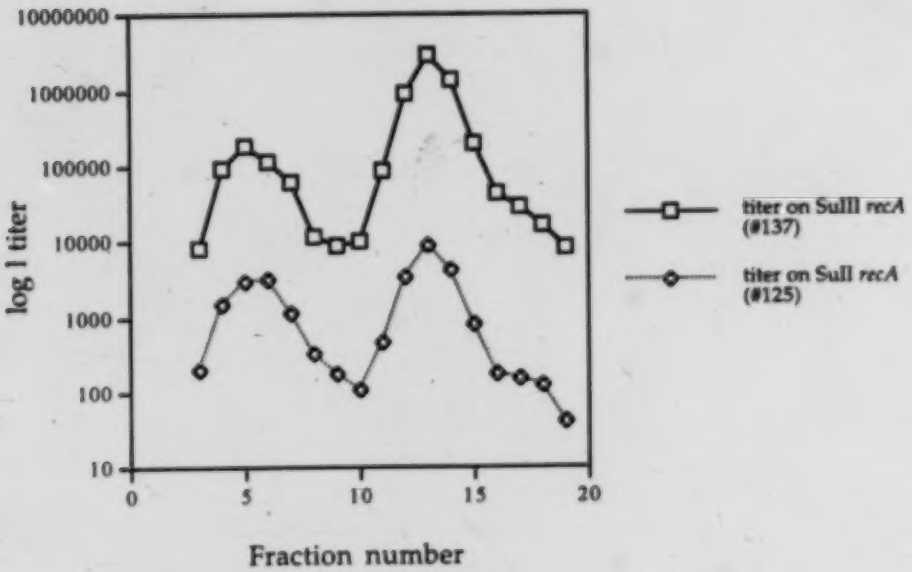
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
1	2580000	300000	12	11	0.8
2	3240000	410000	13		
3	2890000	328000	11		
4	1210000	106000	8.8		
5	347000	27500			
6	339000	26600			
7	221000	20800			
8	216000	16900			
9	6950000	208000			
10	17100000	640000			
11	18700000	658000			
12	5140000	145000			
13	479000	22200			
14	208000	7240			
15	131000	5240			
16	117000	4580			
17	99500	3440			

Experiment #2 - *sbcB recJ*



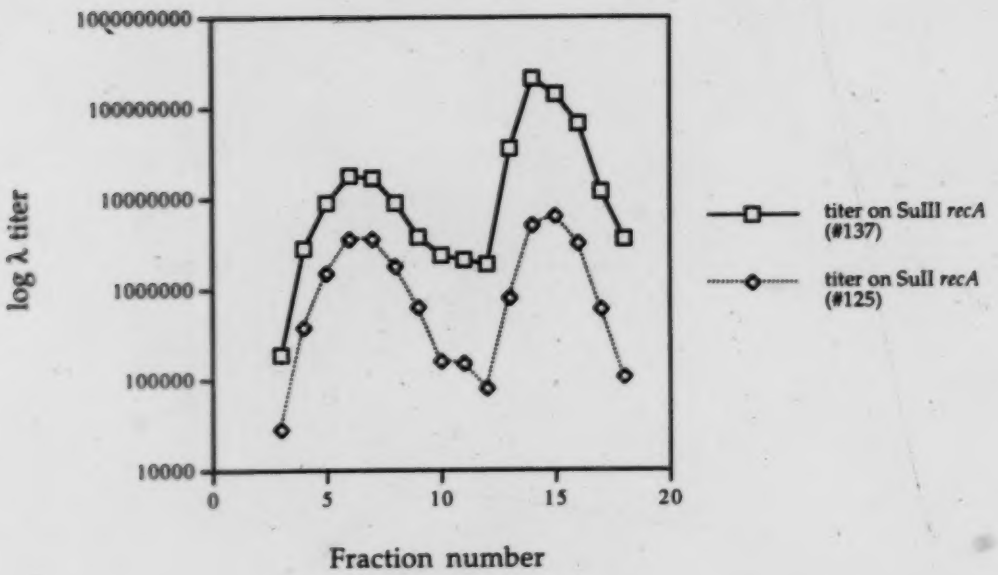
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
1	438000	13400	3.1	3.2	0.2
2	310000	9900	3.2		
3	183000	7550	4.1		
4	66700	1920	2.9		
5	47000	1180	2.5		
6	59500	1170			
7	33700	820			
8	254000	2400			
9	4740000	31900			
10	12200000	92000			
11	8200000	60000			
12	2240000	15200			
13	330000	2010			
14	104000	1110			
15	89700	935			
16	64700	545			
17	54700	520			

Experiment #2 - Δ*xonA recJ*



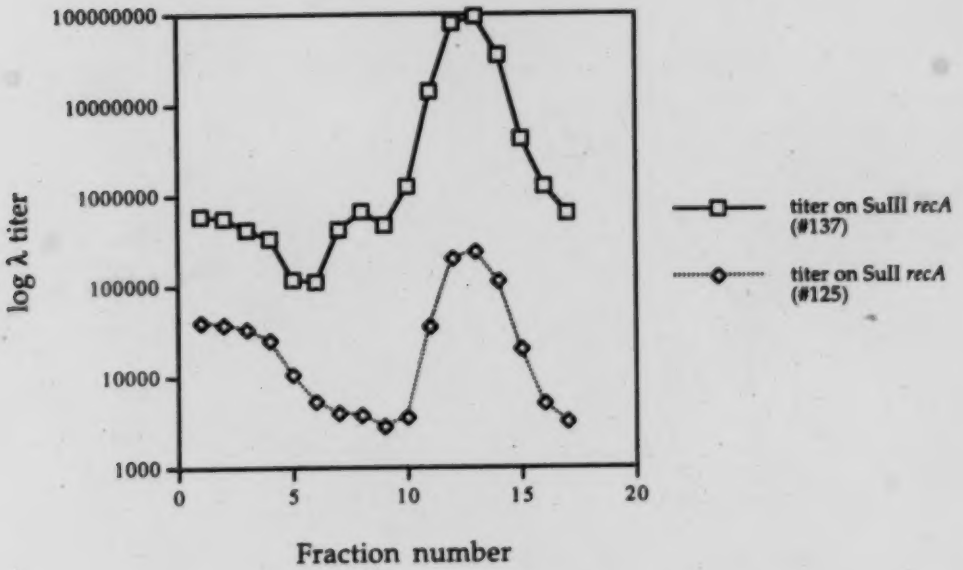
Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
3	8350	205	2.5	2.1	0.2
4	94500	1510	1.6		
5	185000	3000	1.6		
6	115000	3050	2.7		
7	63300	1120	1.8		
8	11800	325	2.8		
9	8800	175	2		
10	10500	110			
11	86000	460			
12	910000	3280			
13	2870000	8650			
14	1350000	4230			
15	197000	800			
16	43200	170			
17	29400	155			
18	17100	125			
19	8400	40			

Experiment #2 - *recD*



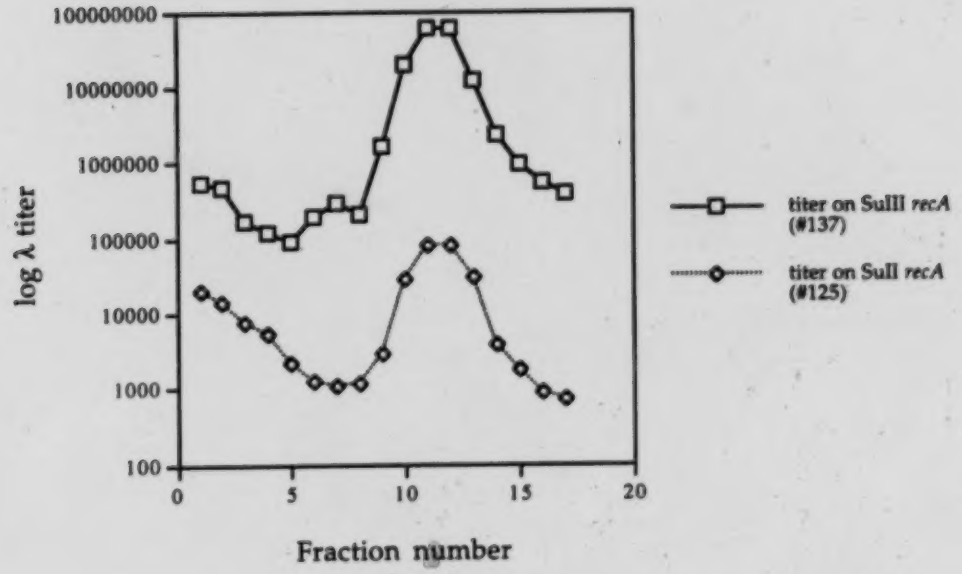
Fraction #	Titer on #137 (int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	±SE
3	197000	29200	15	18	0.9
4	2800000	380000	14		
5	8850000	1550000	18		
6	17700000	3580000	20		
7	17600000	3650000	21		
8	9200000	1860000	20		
9	3830000	643000	17		
10	2380000	159000			
11	2170000	156000			
12	1870000	80000			
13	35300000	830000			
14	212000000	5170000			
15	143000000	6500000			
16	69500000	3260000			
17	11800000	613000			
18	3590000	107000			

Experiment #2 - *sbcB recJ recD*



Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/Int) x 100	Average gen/Int	±SE
1	573000	38500	6.7	7.5	0.4
2	543000	35800	6.6		
3	414000	31800	7.7		
4	332000	24800	7.5		
5	116000	10500	9.1		
6	107000	5330			
7	403000	3900			
8	637000	3600			
9	470000	2740			
10	1250000	3460			
11	13900000	35000			
12	78300000	191000			
13	92700000	229000			
14	35000000	107000			
15	4230000	19300			
16	1200000	4870			
17	633000	3080			

Experiment #2 - Δ*xonA recJ recD*



Fraction #	Titer on #137 (Int)	Titer on #125 (gen)	(gen/int) x 100	Average gen/int	±SE
1	530000	19200	3.6	3.5	0.4
2	460000	13700	3		
3	172000	7580	4.4		
4	120000	5320	4.4		
5	92000	2160	2.3		
6	195000	1210			
7	292000	1060			
8	201000	1200			
9	1700000	2920			
10	19500000	27600			
11	59200000	77000			
12	58300000	78500			
13	12500000	30500			
14	2320000	3760			
15	940000	1740			
16	556000	860			
17	396000	740			

APPENDIX D. LIST OF SINGLE-STRAND EXONUCLEASES IN THIS THESIS STUDY.

Single-strand Exonuclease	Specificity	Gene	Reference
Exonuclease I	single-strand dependent; degrades 3' ends.	*xonA* or *sbcB*	Lehman and Nussbaum (1964)
RecJ	single-strand dependent; degrades 5' ends.	*recJ*	Lovett and Kolodner (1989)
Exonuclease VII	single-strand dependent; degrades 5' and 3' ends.	*xseA*	Chase and Richardson (1974)
Exonuclease III	double-strand dependent; degrades 3' ends.	*xthA*	Kow (1989)

References:

Chase, J. W., and C. C. Richardson, 1974. Exonuclease VII of *Escherichia coli*. Purification and properties. J. Biol. Chem. **249**: 4545-4552.

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Lehman, I. R., and A. L. Nussbaum, 1964. The deoxyribonucleases of *Escherichia coli*. V. On the specificity of exonuclease I (phosphodiesterase). J. Biol. Chem. **239**: 2628-2636.

Lovett, S. T. and R. D. Kolodner, 1989. Identification and purification of a single-strand-DNA-specific exonuclease encoded by the *recJ* gene of *Escherichia coli*. PNAS **86**: 2627-2631.

APPENDIX E. λ MARKERS USED IN THIS THESIS STUDY

λ marker	Relevant properties	Reference/source
Δb1453	deletion from *att* rightward into *gam*	Henderson and Weil (1975)
Δb2	6.0-kb deletion left from *att*	Kellenberger et al. (1960)
Δb516	10% from *att* leftward	Parkinson (1971)
Δb527	deletion of 8.3% of λ left of *att*	Parkinson (1971)
bio1	substitution from *att* rightward, 5% net deletion, unconditional *red⁻gam⁻*	Hradecna and Szbalski (1969)
cIts857	temperature-sensitive in *c*I	Sussman and Jacob (1962)
cII68	unconditional *c*II mutation	Ira Herskowitz
ChiC	Chi site in *c*II	Stahl et al. (1975)
ChiD	leftward-acting *Chi*, right of *nin*	Stahl et al. (1975)
Chi-bio1	*Chi* site in *bio1* substitution	McMilin et al. (1974)
Eam4	amber mutation in *E* (head structural) gene	Murialdo and Siminovitch (1972)
Ets3	temperature-sensitive mutation in *E* (head structural) gene.	Brown and Arber (1964)
gam210	amber mutation in *gam*	Zissler et al. (1971)
imm21	immunity region from phage 21	Campbell (1971)
Mts5	heat-sensitive marker in λ left arm	Brown and Arber (1964)
Δnin5	5% deletion in right arm	Fiandt et al. (1971)
Pam80	amber mutation in *P* (replication gene)	Campbell (1961)
red3	unconditional *red⁻* mutation	Signer and Weil (1968)
red270	amber mutation in *red* gene	Signer and Weil (1968)
Rts129	heat-sensitive mutation in *R* (lysis gene)	Campbell and del Campillo-Campbell (1963)
Sam7	SuIII suppressible, near λ right end	Goldberg and Howe (1969)

References for λ markers

Brown, A., and W. Arber, 1964. Temperature-sensitive mutants of coliphage lambda. Virology **24**: 237-239.

Campbell, A. 1961. Sensitive mutants of bacteriophage lambda. Virology **14**: 22-32.

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Hradecna, Z., and W. Szybalski, 1969. Electron micrographic maps of deletions and substitutions in the genomes of transducing coliphages *λdg* and *λbio*. Virology **38**: 473-477.

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Murialdo, H. and L. Siminovitch, 1972. The morphogenesis of phage lambda. V. Form-determining function of the genes required for the assembly of the head. Virology **48**: 824-835.

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Sussman, R., and F. Jacob, 1962. Sur un systeme de repression thermosensible chez le bacteriophage λ d'*Escherichia coli*. C.R. Acad. Sci. (Paris) **254**: 1517-1519.

Zissler, J., E. Signer, and F. Schaefer, 1971. The role of recombination in growth of bacteriophage lambda I. The gamma gene, pp. 455-468 in *The Bacteriophage Lambda*, edited by A. D. Hershey, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.